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L7 17 L6 AND FC

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L8 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2003:118018 Document No. 138:168835 Targeting of an antigen presenting cell (APC) with a modulator of T cell signalling, such as a Notch ligand, coupled to the MHC class II-binding motif from a superantigen. Bodmer, Mark William; Champion, Brian Robert; McKenzie, Grahame James; Nye, Lucy Emma (Lorantis Limited, UK). PCT Int. Appl. WO 2003012111 A2 20030213, 93 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB3381 20020725. PRIORITY: GB 2001-18155 20010725.

AB The present invention relates to the concept of delivering a modulator of T cell signalling, such as a Notch ligand, to an antigen presenting cell (APC). The targeting approach disclosed uses, for example, the major histocompatibility complex (MHC) class II binding motif from a superantigen coupled to a modulator of the Notch signalling pathway. Superantigens bind both MHC class II mols. and subsets of T cell receptors and thus effectively cross-link APCs to T cells and activate cells

polyclonally. The mol. regions of these mols. that impart T cell receptor (TCR) and MHC class II binding have been defined structurally and have been shown to be distinct regions of the mol. By using the MHC class II binding domain with a modulator of the Notch signalling pathway we can focus the activity of the Notch signalling pathway modulator to the APCs at the site of delivery. Further, the domain lacks toxin activity because it cannot find the T cell receptor to activate T cells. According to one aspect of the present invention there is provided a conjugate comprising a first and a second sequence wherein the first sequence comprises a polypeptide which is capable of binding to an APC, or a polynucleotide encoding therefor, and the second sequence comprises a polypeptide comprising a modulator of a signalling pathway in a T cell or a polynucleotide encoding therefor.

L8 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2002:574952 Document No. 137:139357 Chimeric antibodies comprising **CD64**-binding human **Fc** and heterologous T cell epitopes for stimulating cytotoxic T cell response against pathogens and tumor. Durrant, Linda Gillian; Parsons, Tina; Robins, Adrian (Scancell Limited, UK; Cancer Research Campaign Technology Limited). PCT Int. Appl. WO 2002058728 A2 20020801, 87 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB354 20020128. PRIORITY: GB 2001-2145 20010126.

AB The invention relates to the use of a polypeptide which comprises (i) a first portion comprising the part of human **Fc** which binds to **CD64**, and (ii) a second portion comprising one or more heterologous T cell epitopes for stimulating cytotoxic and helper T cell response. The polypeptide may be an antibody which may be used to stimulate an cytotoxic T cell response against pathogens and tumor cells in patients in need of such treatment.

L8 ANSWER 3 OF 17 MEDLINE on STN
2001669005 Document Number: 21571693. PubMed ID: 11714794. Receptor modulation by **Fc** gamma RI-specific **fusion proteins** is dependent on receptor number and modified by IgG. Guyre C A; Keler T; Swink S L; Vitale L A; Graziano R F; Fanger M W. (Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756, USA.) JOURNAL OF IMMUNOLOGY, (2001 Dec 1) 167 (11) 6303-11. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The high-affinity IgG receptor, FcgammaRI (**CD64**), is constitutively expressed exclusively on professional APCs. Human FcgammaRI binds monomeric IgG with high affinity and is, therefore, saturated in vivo. The binding of IgG to FcgammaRI causes receptor recycling, while Abs that cross-link FcgammaRI cause rapid down-modulation of surface FcgammaRI. Because studies performed in the absence of ligand may not be representative of FcgammaRI modulation in vivo, we investigated the ability of FcgammaRI-cross-linking Abs and non-cross-linking derivatives to modulate FcgammaRI in the presence and absence of ligand. In the absence of ligand mAb H22 and wH22xeGFP, an enhanced green fluorescent protein (eGFP)-labeled **fusion protein** of H22, cross-linked and rapidly down-modulated surface FcgammaRI on the human myeloid cell line, U937, and its high FcgammaRI-expressing subclone, 10.6. This effect was dependent on the concentration of **fusion protein** and the level of FcgammaRI expression and correlated with internalization of both wH22xeGFP and FcgammaRI, itself, as assessed by confocal microscopy. A single-chain Fv version, sFv22xeGFP, which does not cross-link FcgammaRI, was unable to modulate FcgammaRI in the absence

of IgG. However, if ligand was present, treatment with either monovalent or cross-linking **fusion protein** led to intracellular receptor accumulation. These findings suggest at least two alternate mechanisms of internalization that are influenced by ligand and demonstrate the physiologic potential of FcgammaRI to transport a large antigenic load into APCs for processing. These studies may lead to the development of better FcgammaRI-targeted vaccines, as well as therapies to down-modulate FcR involved in autoimmune diseases.

L8 ANSWER 4 OF 17 MEDLINE on STN

2001213273 Document Number: 21103279. PubMed ID: 11160307. Colocalization of **Fc** gamma RI-targeted antigen with class I MHC: implications for antigen processing. Guyre C A; Barreda M E; Swink S L; Fanger M W. (Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756, USA.) JOURNAL OF IMMUNOLOGY, (2001 Feb 15) 166 (4) 2469-78. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The high-affinity receptor for IgG (**CD64** or FcgammaRI) is constitutively expressed exclusively on professional APCs (monocytes, macrophages, and dendritic cells). When Ag is targeted specifically to FcgammaRI, Ag presentation is markedly enhanced, although the mechanism of this enhancement is unknown. In an effort to elucidate the pathways involved in FcgammaRI targeting, we developed a model targeted Ag using enhanced green fluorescent protein (eGFP). This molecule, wH22xeGFP, consists of the entire humanized anti-FcgammaRI mAb H22 with eGFP genetically fused to the C-terminal end of each CH3 domain. wH22xeGFP binds within the ligand-binding region by its **Fc** end, as well as outside the ligand-binding region by its Fab ends, thereby cross-linking FcgammaRI. Confocal microscopy studies revealed that wH22xeGFP was rapidly internalized by the high-FcgammaRI-expressing cell line U937 10.6, but did not associate with intracellular proteins Rab4, Rab5a, or Lamp-1, suggesting that the targeted **fusion protein** was not localized in early endosomes, recycling vesicles, or lysosomes. Interestingly, wH22xeGFP was found colocalized with intracellular MHC class I, suggesting that FcgammaRI-targeted Ags may converge upon a class I processing pathway. These data are in agreement with studies in the mouse showing that FcgammaRI targeting can lead to Ag-specific activation of cytotoxic T cells. Data obtained from these studies should lead to a better understanding of how Ags targeted to FcgammaRI are processed and under what conditions they lead to presentation of antigenic peptides in MHC class I, as a foundation for the use of FcgammaRI-targeted Ags as vaccines.

L8 ANSWER 5 OF 17 MEDLINE on STN

2001074799 Document Number: 20569377. PubMed ID: 11119616. Functional and selective targeting of adenovirus to high-affinity Fcgamma receptor I-positive cells by using a bispecific hybrid adapter. Ebbinghaus C; Al-Jaibaji A; Operschall E; Schoffel A; Peter I; Greber U F; Hemmi S. (Institute of Molecular Biology, University of Zurich, CH-8057 Zurich, Switzerland.) JOURNAL OF VIROLOGY, (2001 Jan) 75 (1) 480-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Adenovirus (Ad) efficiently delivers its DNA genome into a variety of cells and tissues, provided that these cells express appropriate receptors, including the coxsackie-adenovirus receptor (CAR), which binds to the terminal knob domain of the viral capsid protein fiber. To render CAR-negative cells susceptible to Ad infection, we have produced a bispecific hybrid adapter protein consisting of the amino-terminal extracellular domain of the human CAR protein (CARex) and the **Fc** region of the human immunoglobulin G1 protein, comprising the hinge and the CH2 and CH3 regions. CARex-**Fc** was purified from COS7 cell supernatants and mixed with Ad particles, thus blocking Ad infection of CAR-positive but **Fc** receptor-negative cells. The functionality of the CARex domain was further confirmed by successful immunization of mice with CARex-**Fc** followed by selection of a monoclonal

anti-human CAR antibody (E1-1), which blocked Ad infection of CAR-positive cells. When mixed with Ad expressing eGFP, CARex-**Fc** mediated an up to 250-fold increase of transgene expression in CAR-negative human monocytic cell lines expressing the high-affinity Fcgamma receptor I (**CD64**) but not in cells expressing the low-affinity Fcgamma receptor II (CD32) or III (CD16). These results open new perspectives for Ad-mediated cancer cell vaccination, including the treatment of acute myeloid leukemia.

L8 ANSWER 6 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2002:129947 Document No.: PREV200200129947. Membrane targeting of dimeric interleukin (IL)-2-immunoglobulin G1 induces cytotoxic T cell activity against autologous human acute myeloid leukemia (AML) blasts and prevents T cell apoptosis. Notter, Michael [Reprint author]; Erben, Ulrike [Reprint author]; Bittroff-Leben, Alexandra [Reprint author]; Thiel, Eckhard [Reprint author]. Hematology, Oncology and Transfusion Medicine, Benjamin Franklin Medical Center, Free University, Berlin, Germany. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 122a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Tumor-induced T cell anergy is a limiting factor for the induction of an autologous transplant rejection-type antileukemic immune reaction in immunotherapy development. We tested the hypothesis that targeting IL-2 to the membrane of AML blasts and of immune effector cells induces a proliferative and cytotoxic T cell (CTL) response. Fusing the IL-2 gene to the hinge-, CH2-, and CH3-domain of human immunoglobulin (Ig) G1, and eukaryotic expression resulted in a dimeric IL-2-IgG1 molecule of 125 kDa. IL-2-IgG1 specifically bound to the high-affinity **Fc**-gamma receptor (R) I (**CD64**) of AML blasts. In the tumor membrane-bound state, IL-2-IgG1 increased proliferation of autologous remission T cells, and, in this respect, was superior to limiting concentrations of soluble IL-2. AML-M5 blasts coated with IL-2-IgG1, and CD8-IgG1 for control, were used as stimulator cells for autologous T cells. Despite the absence of soluble IL-2, IL-2-IgG1 presented on tumor cells on days 0 and 7 of culture, was sufficient to induce CTL activity on day 14 redirected against AML blasts by OKT3 present during the effector phase (21.5+-3.2% versus 7.8+-3.1% specific 51chromium-release at an effector:target ratio of 10:1). IL-2 R binding affinity of IL-2-IgG1 was 100-fold higher compared to IL-2. IL-2-dependent CTLL-16 cells were preincubated with saturating amounts of IL-2-IgG1 and monomeric IL-2. Following removal of excess cytokines by extensive washing, T cells coated with IL-2 did no longer incorporate 3H-thymidine after 24 hours, whereas IL-2-IgG1-pretreated CTLL-16 cells continued to proliferate (905+-72 versus 54.830+-4.652 cpm). Targeting IL-2-IgG1 but not IL-2 to CTLL 16 cells maintained their membrane asymmetry during this 24-hour culture period based on measuring phosphatidylserine exposure by annexin V binding using flow cytometry (13% versus 78% annexin V-positive cells). Furthermore, identical culture conditions followed by gel electrophoretic analysis revealed laddering of genomic DNA typical for apoptosis in the case of IL-2-pretreated CTLL-16 cells. In contrast, IL-2-IgG1 completely inhibited DNA fragmentation in this system indicating that its T cell-supporting activity relates to prevention of apoptosis. In conclusion, structural alterations of IL-2-IgG1 translated into novel biological effects creating potential to increase the immunogenicity of AML blasts for autologous T cells.

L8 ANSWER 7 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:46827 Document No.: PREV200000046827. Dual signal targeting using a novel stem cell factor (SCF) 175-interleukin (IL)-2-myc His **fusion protein** and CD3 antibody against aberrant antigens of human acute myeloid leukemia (AML) blasts selectively stimulates T cells. Notter, Michael [Reprint author]; Erben, Ulrike [Reprint author]; Leben, Alexandra [Reprint author]; Bochert, Gudrun [Reprint author]; Thiel, Eckhard

[Reprint author]. Hematology, Oncology and Transfusion Medicine, Universitaetsklinikum Benjamin Franklin, Berlin, Germany. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 79a. print.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L8 ANSWER 8 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:46828 Document No.: PREV200000046828. Targeting of a B7-1(CD80)IgG **fusion protein** to acute myeloid leukemia (AML) blasts increases their costimulatory activity for autologous remission T cells. Willinger, Tim [Reprint author]; Thiel, Eckhard [Reprint author]; Notter, Michael [Reprint author]. Abteilung fuer Haematologie und Onkologie, Universitaetsklinikum Benjamin Franklin, Berlin, Germany. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 79a. print.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L8 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2000:908511 Document No. 135:44922 Targeting HIV-1 gp120 to the high affinity **FC** receptor (**FC**.gamma.RI, **CD64**) on myeloid antigen presenting cells: implications for enhancing vaccine responses. Howell, Alexandra L.; Thacker, Tara N.; Li, Fang; Fiering, Steve; Graziano, Robert F.; Goldstein, Joel; Fanger, Michael W. (V.A. Medical Center, VT, 05009, USA). Current Topics in Virology, 1, 61-70 (English) 1999. CODEN: CTVUAG. Publisher: Research Trends.
AB We prepd. a **fusion protein** contg. a humanized monoclonal antibody (mAb), mAb H22, with specificity for the human high affinity **Fc** receptor for IgG (**Fc**.gamma.RI, **CD64**), fused to HIV-1 gp120. This **fusion protein** construct was produced by joining the cDNA for the full length H22 heavy chain gene in frame to the cDNA for gp120. This construct, which also expressed a selectable marker, was stably transfected into a murine myeloma cell line that expressed the previously transfected H22 kappa light chain. The resulting **fusion protein**, (H22 .times. gp120), was secreted from the myeloma cell line and was purified by affinity chromatog. Flow cytometric anal. revealed that H22 .times. gp120 bound with high affinity via the Fab portion of H22 to **CD64** expressed on monocytes and macrophages from both humans and human **CD64**-expressing transgenic mice. Western blot anal. revealed that the 390 kDa **fusion protein** reacted with both anti-human IgG and anti-gp120 mAbs. Incubation of a monocyte cell line with this **fusion protein** at 37.degree.C resulted in internalization of the complex as detd. by flow cytometric anal. Immunization of human **CD64** transgenic mice with the purified H22 .times. gp120 **fusion protein** induced higher titers of anti-gp120 serum antibodies compared to immunization of non-transgenic littermates. Targeting gp120 to **CD64**-expressing antigen presenting cells (APC) in vivo may augment immune responses and enhance protective immunity.

L8 ANSWER 10 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1999:94795 Document No.: PREV199900094795. Targeting of A B7-1 (CD80) immunoglobulin G1 (IgG) **fusion protein** to acute myeloid leukemia (AML) blasts increases their costimulatory activity for autologous remission T cells. Willinger, T.; Thiel, E.; Notter, M.. Dep. Hematol. Oncol., Lab. Applied Cell. Mol. Immunol., Universitaetsklin. Benjamin Franklin, FU Berlin, Germany. Annals of Hematology, (1998) Vol. 77, No. SUPPL. 2, pp. S112. print.
Meeting Info.: Annual Congress of the German and Austrian Societies of Hematology and Oncology. Frankfurt, Germany. October 25-28, 1998. Austrian Society of Hematology and Oncology; German Society of Hematology and

Oncology.
ISSN: 0939-5555. Language: English.

L8 ANSWER 11 OF 17 MEDLINE on STN
97146059 Document Number: 97146059. PubMed ID: 8993006. Cytolytic and cytostatic properties of an anti-human **Fc** gammaRI (**CD64**) x epidermal growth factor bispecific **fusion protein**.
Goldstein J; Graziano R F; Sundarapandiyan K; Somasundaram C; Deo Y M. (Medarex, Inc., Annandale, NJ 08801, USA.) JOURNAL OF IMMUNOLOGY, (1997 Jan 15) 158 (2) 872-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB A bispecific **fusion protein** (H22-EGF) that binds simultaneously to the epidermal growth factor receptor (EGF-R) and to the high affinity receptor for the **Fc** portion of human IgG, **Fc** gammaRI (**CD64**), has been successfully constructed and expressed. For this construction, genomic DNA encoding the Fd fragment of humanized anti-**Fc** gammaRI mAb, H22, which binds **Fc** gammaRI at an epitope that is distinct from the **Fc** binding site, was fused to cDNA encoding human epidermal growth factor (EGF), a natural ligand for EGF-R. The resulting H22Fd-EGF-expressing vector was transfected into a myeloma cell line that was transfected previously with a vector containing DNA encoding the H22 kappa-light chain. SDS-PAGE analysis of purified H22-EGF demonstrated that the **fusion protein** was secreted predominantly as H22Fab'-EGF monomer (approximately 55 kDa), even though a free Cys residue exists in the hinge region of the H22 Fab' component. Using a novel bispecific flow cytometry-binding assay, we demonstrated that the purified bispecific **fusion protein**, H22-EGF, was able to bind simultaneously to soluble **Fc** gammaRI and EGF-R-expressing cells. H22-EGF inhibited the growth of EGF-R-overexpressing tumor cells and mediated dose-dependent cytotoxicity of these cells in the presence of **Fc** gammaRI-bearing cytotoxic effector cells. These results suggest that this **fusion protein** may have therapeutic utility for EGF-R-overexpressing malignancies.

L8 ANSWER 12 OF 17 MEDLINE on STN
1998098148 Document Number: 98098148. PubMed ID: 9435859. Increased potency of **Fc**-receptor-targeted antigens. Guyre P M; Graziano R F; Goldstein J; Wallace P K; Morganelli P M; Wardwell K; Howell A L. (Department of Physiology, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA.. paul.guyre@dartmouth.edu) . CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 146-8. Ref: 13. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB A major challenge for using native and modified T cell epitopes to induce or suppress immunity relates to achieving efficient uptake and processing by antigen-presenting cells (APC) in vivo. IgG **Fc** receptors, which are expressed constitutively by professional APC including monocytes and dendritic cells, have long been known to mediate antigen uptake in a manner leading to efficient T cell activation. We have previously demonstrated enhanced presentation of antigenic and antagonistic peptides by targeting them to the type I **Fc** receptor for IgG (**Fc** gamma RI, **CD64**) on human monocytes. In the present report we review the literature suggesting that **CD64**-targeted antigens are likely to be effective in vivo, and present data demonstrating enhanced immunogenicity in **CD64** transgenic mice of a **fusion protein** that combines the specificities of HIV gp120 and the humanized anti-**CD64** monoclonal antibody H22. Overall, these studies suggest that targeting antigens to **CD64** represents an effective approach to enhancing the effectiveness of vaccines in vivo.

L8 ANSWER 13 OF 17 MEDLINE on STN
97060283 Document Number: 97060283. PubMed ID: 8903318. F(c)gammaRI-targeted **fusion prot ins** result in efficient presentation by human monocytes of antigenic and antagonist T

cell epitopes. Liu C; Goldstein J; Graziano R F; He J; O'Shea J K; Deo Y; Guyre P M. (Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1996 Nov 1) 98 (9) 2001-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB A major challenge for using native or modified T cell epitopes to induce or suppress immunity relates to poor localization of peptides to antigen presenting cells (APCs) in vivo. In this study, we demonstrate enhanced presentation of antigenic and antagonistic peptides by targeting them to the type I **Fc** receptor for IgG (F(c)gammaRI, **CD64**) on human monocytes. A Th epitope of tetanus toxoid, TT830, and the antagonistic peptide for TT830, TT833S, were genetically grafted into the constant region of the heavy chain of the humanized anti-**CD64** mAb 22 and expressed as monovalent **fusion proteins**, Fab22-TT830 and Fab22-TT833S. These **CD64**-targeted peptides were up to 1,000- and 100-fold more efficient than the parent peptides for T cell stimulation and antagonism, respectively, suggesting that such **fusion proteins** could effectively increase the delivery of peptides to APCs in vivo. Moreover, the F(c)gammaRI-targeted antagonistic peptide inhibited proliferation of TT830-specific T cells even when APCs were first pulsed with native peptide, a situation comparable with that which would be encountered in vivo when attempting to ameliorate an autoimmune response. These data suggest that targeted presentation of antagonistic peptides could lead to promising Ag-specific therapies for T cell-mediated autoimmune diseases.

L8 ANSWER 14 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1996:310083 Document No.: PREV199699032439. Presentation of agonist and antagonist T cell epitopes targeted to **Fc**-gamma-RI on human monocytes using anti-**Fc**-gamma-RI antibody-based **fusion proteins**. Liu, C.; Graziano, R. F.; Goldstein, J.; He., J.; O'Shea, J.; Guyre, P. M.. Physiol. Dep., Dartmouth Med. Sch., Lebanon, NJ 03756, USA. FASEB Journal, (1996) Vol. 10, No. 6, pp. A1455. Meeting Info.: Joint Meeting of the American Society for Biochemistry and Molecular Biology, the American Society for Investigative Pathology and the American Association of Immunologists. New Orleans, Louisiana, USA. June 2-6, 1996.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L8 ANSWER 15 OF 17 MEDLINE on STN
95138530 Document Number: 95138530. PubMed ID: 7836769. Interaction of human monocyte **Fc** gamma receptors with rat IgG2b. A new indicator for the **Fc** gamma RIIa (R-H131) polymorphism. Haagen I A; Geerars A J; Clark M R; van de Winkel J G. (Department of Immunology, University Hospital Utrecht, The Netherlands.) JOURNAL OF IMMUNOLOGY, (1995 Feb 15) 154 (4) 1852-60. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Rat mAbs receive considerable interest for immunologic intervention in man. The rat IgG2b isotype has previously been found to be optimally active both in vivo and in vitro. We found that both a rat IgG2b CD3 mAb and a monovalent hybrid rat IgG2b-mouse IgG1 bispecific Ab triggered T cell activation in PBMC. Inhibition analyses with mAb blocking different human IgG **Fc** receptors (**Fc** gamma R) showed a dimorphic pattern. In donors expressing an **Fc** gamma RIIa-R/R131 allotype (previously defined on the basis of interaction with mouse (m) IgG1 as "high responder") anti-**Fc** gamma RI mAb 197 inhibited rat IgG2b induced T cell mitogenesis almost completely. In **Fc** gamma RIIa-H/H131 ("low responder" allotype) donors, however, both anti-**Fc** gamma RI mAb 197 and anti-**Fc** gamma RII mAb IV.3 were essential for optimal inhibition of mitogenesis. T cell proliferation experiments performed with the use of **Fc** gamma R-transfected fibroblasts as accessory cells showed the high affinity **Fc** gamma RIa (**CD64**) to interact with both rat IgG2b and rat IgG2b-mIgG1 hybrid CD3 mAb. The use of the two types of **Fc** gamma RIIa (CD32)-transfectants instead showed rat IgG2b CD3 mAb to interact solely

with the IIa-H/H131 allotype. Interestingly, rat IgG2b-mIgG1 hybrid mAb did not interact effectively with this low affinity **Fc** gamma R. This suggests a requirement for only one rat IgG2b H chain for **Fc** gamma RIa-mediated binding, whereas two identical H chains seem to be necessary for proper interaction with **Fc** gamma RIIa. Ab-sensitized RBC-rosette experiments performed with the use of a rat IgG2b anti-NIP mAb confirmed the interaction pattern observed with rat CD3 mAb, supporting the phenomena to be isotype-, and not mAb-, dependent. These analyses point to a unique reactivity pattern for rat IgG2b Abs, interacting both with the high affinity **Fc** gamma RIa in all donors and **Fc** gamma RIIa of individuals expressing the IIa-H131 allotype.

L8 ANSWER 16 OF 17 MEDLINE on STN

96129466 Document Number: 96129466. PubMed ID: 8581368. A human **Fc** gamma RI/**CD64** transgenic model for in vivo analysis of (bispecific) antibody therapeutics. Heijnen I A; Van de Winkel J G. (Department of Immunology, University Hospital Utrecht, The Netherlands.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 351-6. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB The human high-affinity IgG receptor, hFc gamma RI (**CD64**), is exclusively expressed on myeloid cells, where it serves an important role as a (cytotoxic) trigger molecule. To establish an in vivo model for analysis of the role of hFc gamma RI in immunity, we developed a novel transgenic mouse model. The human **Fc** gamma RIA gene, with endogenous regulatory sequences, was used to generate two lines of transgenic FVB/N mice. Immunohistochemical and flow cytometric studies showed that hFc gamma RI expression was restricted to myeloid cells. Monocytes, macrophages, and polymorphonuclear neutrophils (PMN) expressed physiologic hFc gamma RI levels, whereas lymphocytes and mast cells lacked expression. Human **Fc** gamma RI expression was regulated in vivo by the cytokines IFN-gamma (exactly as in humans) and IL-10. The transgenic receptor proved functional and bound human tumor cells via anti-hFc gamma RI-based bispecific antibodies. hFc gamma RI could, furthermore, be efficiently targeted in vivo by **CD64** antibodies. These data demonstrate that the hFc gamma RI transgenic mouse model closely parallels the situation in humans. This mouse model seems useful for in vivo evaluation of the therapeutic potential of novel bispecific reagents in tumor and infection models.

L8 ANSWER 17 OF 17 MEDLINE on STN

92113247 Document Number: 92113247. PubMed ID: 1530954. Characterization of IgG FcR-mediated proliferation of human T cells induced by mouse and human anti-CD3 monoclonal antibodies. Identification of a functional polymorphism to human IgG2 anti-CD3. Parren P W; Warmerdam P A; Boeije L C; Capel P J; van de Winkel J G; Aarden L A. (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.) JOURNAL OF IMMUNOLOGY, (1992 Feb 1) 148 (3) 695-701. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB T cell activation induced by mouse anti-CD3 mAb has shown to be dependent on the Ig isotype of these antibodies. A study of isotype dependency of human antibodies, however, seems more relevant to human effector systems, especially in view of the availability of humanized antibodies for clinical applications. We constructed a panel of mouse and mouse/human chimeric anti-CD3 mAb, which differ only in their CH region and hence have identical binding sites and affinity. By using these antibodies, we now studied their ability to induce T cell proliferation in human PBMC and analyzed the classes of IgG FcR involved in these responses. The human (h)IgG1, hIgG3, and hIgG4, as well as mouse (m)IgG2a and mIgG3 anti-CD3 mAb induced an **Fc** gamma RI (**CD64**)-dependent T cell proliferation in all donors. Activation with hIgG2 and mIgG1 anti-CD3 mAb was observed to be mediated via the low affinity **Fc** gamma RII (CD32). It was found that leukocytes in a normal donor population display a functional polymorphism with respect to hIgG2 anti-CD3 responsiveness. This polymorphism was found to be inversely related to the previously

defined **Fc** gamma RII-polymorphism to mIgG1 anti-CD3 mAb. Monocytes expressing the **Fc** gamma RII mIgG1 low responder (LR) allele support hIgG2 anti-CD3 induced T cell proliferation efficiently, whereas cells homozygous for the **Fc** gamma RII mIgG1 high responder (HR) allele do not. This observation could be confirmed in T cell activation studies using hFc gamma RIIa-transfected mouse fibroblasts, expressing either the mIgG1 anti-CD3 HR or LR **Fc** gamma RII-encoding cDNA.

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=> d his

     (FILE 'HOME' ENTERED AT 14:04:34 ON 26 NOV 2003)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:04:54 ON
     26 NOV 2003
L1        151321 S FUSION PROTEIN
L2          4806 S L1 AND B CELL
L3             0 S L2 AND CD64
L4            58 S L1 AND CD64
L5             0 S L4 AND T HELPER EPITOPE
L6            24 DUP REMOVE L4 (34 DUPLICATES REMOVED)
L7            17 S L6 AND FC
L8            17 DUP REMOVE L7 (0 DUPLICATES REMOVED)

=> s l1 and Fc fusion
L9          2016 L1 AND FC FUSION

=> s l1 and CD79
L10            3 L1 AND CD79

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11            3 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> d l11 1-3 cbib abs

L11  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:417584   Document No. 139:5639  Anti-CD30 antibody-cytotoxic agent
     conjugates for treating non-cancer immunological disorders.  Law,
     Che-Leung; Klussman, Kerry; Wahl, Alan F.; Senter, Peter; Doronina,
     Svetlana; Toki, Brian (Seattle Genetics, Inc., USA).  PCT Int. Appl. WO
     2003043583 A2 20030530, 194 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM,
     AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK,
     DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,
     KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
     MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ,
     MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES,
     FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG,
     TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO 2002-US37223 20021120.
     PRIORITY: US 2001-PV331750 20011120.
AB   The present invention relates to methods for the treatment of immunol.
     disorders other than cancer, comprising administering proteins
     characterized by their ability to bind to CD30 and exert a cytostatic or
     cytotoxic effect on an activated lymphocyte.  Such proteins include
     monoclonal antibodies AC10 and HeFi1, AC10 and HeFi-1 derivs., and
     antibodies that compete with AC10 and HeFi-1 for binding to CD30.  Other
     such proteins include multivalent anti-CD30 antibodies and anti-CD30
     antibodies conjugated to cytotoxic agents.  These antibody conjugates are
     used for treating immunol. disorders such as autoimmune diseases,
     allergies, chronic inflammatory rections and graft vs. host diseases.

L11  ANSWER 2 OF 3     MEDLINE on STN
2002645523  Document Number: 22271083.     PubMed ID: 12384401.    The
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alternative transcript of CD79b is overexpressed in B-CLL and inhibits signaling for apoptosis. Cragg Mark S; Chan H T Claude; Fox Mathew D; Tutt Alison; Smith Aimee; Oscier David G; Hamblin Terry J; Glennie Martin J. (Tenovus Research Laboratory, Cancer Sciences Division, University of Southampton School of Medicine, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK.. msc@soton.ac.uk) . BLOOD, (2002 Nov 1) 100 (9) 3068-76. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB The B-cell receptor (BCR) for antigen is composed of surface immunoglobulin (sIg), which provides antigen specificity, and a noncovalently associated signaling unit, the CD79a/b heterodimer. Defects in **CD79** can influence both BCR expression and signaling and may explain why cells from certain malignancies, such as B-chronic lymphocytic leukemia (B-CLL), often express diminished and inactive BCR. Recently, an alternative transcript of CD79b (DeltaCD79b) has been reported that is up-regulated in B-CLL and may explain this diminished BCR expression. Here we assess the expression of DeltaCD79b in B-CLL and other lymphoid malignancies and investigate its function. High relative expression of DeltaCD79b was confirmed in most cases of B-CLL and found in 6 of 6 cases of splenic lymphomas with villous lymphocytes (SLVLs) and hairy cell leukemia. In a range of Burkitt lymphoma cell lines, expression of DeltaCD79b was relatively low but correlated inversely with the ability of the BCR to signal apoptosis when cross-linked by antibody (Ab). Interestingly, when Ramos-EHRB cells, which express low DeltaCD79b, were transfected with this transcript, they were transformed from being sensitive to anti-Fcmu-induced apoptosis to being highly resistant. Although DeltaCD79b was expressed as protein, its overexpression did not reduce the level of cell surface BCR. Finally, we showed that the inhibitory activity of DeltaCD79b depended on an intact leader sequence to ensure endoplasmic reticulum (ER) trafficking and a functional signaling immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic tail. These results point to DeltaCD79b being a powerful modulator of BCR signaling that may play an important role in normal and malignant B cells.

L11 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN

2000:756855 Document No. 133:318803 Synthetic signal transducing proteins using motifs associated with receptor binding and activation. Lawson, Alastair David Griffiths; Finney, Helene Margaret (Celltech Therapeutics Limited, UK). PCT Int. Appl. WO 2000063372 A1 20001026, 74 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB1456 20000417. PRIORITY: GB 1999-8807 19990416.

AB The invention relates to synthetic signalling mols., which are based on sequences derived from primary signalling motifs such as Ig tyrosine receptor-based activation motifs (ITAMs). The use of such signalling mols. within chimeric receptor proteins allows one to tailor the level of intracellular signalling mediated by the chimeric receptor. Proteins contg., and nucleic acids encoding, such synthetic signalling mols. suitable for use in medicine, are described.

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=> s l1 and CD20
L12          160 L1 AND CD20

=> dup remove l12
PROCESSING COMPLETED FOR L12
L13          112 DUP REMOVE L12 (48 DUPLICATES REMOVED)

=> s l13 and T helper epitope
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L14 0 L13 AND T HELPER EPITOPE

=> s CD20 fusion protein
L15 7 CD20 FUSION PROTEIN

=> dup remove l15
PROCESSING COMPLETED FOR L15
L16 3 DUP REMOVE L15 (4 DUPLICATES REMOVED)

=> d l16 1-3 cbib abs

L16 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
2003064199 Document Number: 22462454. PubMed ID: 12573619. An oncolytic measles virus engineered to enter cells through the CD20 antigen. Bucheit Amanda D; Kumar Shaji; Grote Deanna M; Lin Yukang; von Messling Veronika; Cattaneo Roberto B; Fielding Adele K. (Mayo Clinic Molecular Medicine Program, 200 First Street SW, Rochester, Minnesota 55902, USA.) MOLECULAR THERAPY, (2003 Jan) 7 (1) 62-72. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.
AB We have earlier shown that attenuated measles virus (MV) has therapeutic potential as a replicating oncolytic virus in models of non-Hodgkin's lymphoma (NHL). In the current study, we investigated whether we could obtain replicating MVs capable of entering CD20(+) target cells through an interaction between a single-chain (scFv) anti-CD20 antibody and the CD20 antigen, a target of considerable clinical relevance in NHL. We replaced the H envelope glycoprotein of MV by an H-scFv anti-**CD20 fusion protein** with and without a protease-cleavable linker. Biochemical analysis of purified virions confirmed that the modified H proteins were incorporated into the viral particles with efficiency similar to unmodified H. Experiments employing CHO cells and CHO cells expressing human CD20 indicated that the MVH alpha CD20 viruses were able to replicate well in CHOCD20 but not CHO cells. MVH alpha CD20 or a nonmodified control MV were administered systemically to immunodeficient mice bearing bilateral human tumor xenografts, one side with and the other side without CD20 expression. Growth of CD20(+) tumors was retarded by MVH alpha CD20 as compared with the control virus. The viruses had equivalent effects on the CD20(-) tumors. Thus we have demonstrated that the entry of a replicating oncolytic virus can be mediated through an interaction between a highly clinically relevant single-chain antibody and its target antigen, and we have shown that this interaction enhances in vivo oncolytic activity.

L16 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 2
2002:455929 Document No.: PREV200200455929. Pretarget radioimmunotherapy (RIT) with anti-**CD20 fusion protein** in patients with non-Hodgkin's lymphoma (NHL). Meredith, R. [Reprint author]; Shen, S.; Breitz, H.; Fisher, D.; Goris, M.; Knox, S.; Hankins, J.; Vose, J.; Picozzi, V.. University of Alabama, Birmingham, AL, USA. Journal of Nuclear Medicine, (May, 2002) Vol. 43, No. 5 Supplement, pp. 116P-117P. print.
Meeting Info.: 49th Annual Meeting of the Society of Nuclear Medicine. Los Angeles, CA, USA. June 15-19, 2002.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L16 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
2002:157379 Document No. 136:293206 Multimerization of a chimeric anti-CD20 single-chain Fv-Fc fusion protein is mediated through variable domain exchange. Wu, Anna M.; Tan, Giselle J.; Sherman, Mark A.; Clarke, Patrick; Olafsen, Tove; Forman, Stephen J.; Raubitschek, Andrew A. (Dep. of Mol. Biol., Beckman Res. Inst. of the City of Hope, Duarte, CA, 91010, USA). Protein Engineering, 14(12), 1025-1033 (English) 2001. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.
AB A series of single-chain anti-CD20 antibodies was produced by fusing single-chain Fv (scFv) with human IgG1 hinge and Fc regions, designated

scFv-Fc. The anti-CD20 scFv-Fc retained its specific binding to CD20-pos. cells and was active in mediating complement-dependent cytolysis. However, the purified scFv-Fc included multimeric as well as monomeric components as revealed in the size-exclusion HPLC anal. Variant scFv-Fc were constructed incorporating four different hinges between the scFv and Fc regions, or three different linkers in the scFv domain. All formed multimers, with the highest level of multimerization obsd. in the scFv-Fc with the shortest linker (8 aa). The structural anal. of the scFv-Fc constructed with 18 or 8 aa linkers by pepsin or papain cleavage indicated that the proteins contained a form in which scFv units had cross-paired to form a "diabody". Such a domain exchange or cross-pairing appears to be the basis of the obsd. multimerization.

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=> s l1 and CD64
L17          58 L1 AND CD64

=> dup remove l17
PROCESSING COMPLETED FOR L17
L18          24 DUP REMOVE L17 (34 DUPLICATES REMOVED)

=> d l18 1-24 cbib abs
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L18 ANSWER 1 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN
2003:118018 Document No. 138:168835 Targeting of an antigen presenting cell (APC) with a modulator of T cell signalling, such as a Notch ligand, coupled to the MHC class II-binding motif from a superantigen. Bodmer, Mark William; Champion, Brian Robert; McKenzie, Grahame James; Nye, Lucy Emma (Lorantis Limited, UK). PCT Int. Appl. WO 2003012111 A2 20030213, 93 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB3381 20020725. PRIORITY: GB 2001-18155 20010725.

AB The present invention relates to the concept of delivering a modulator of T cell signalling, such as a Notch ligand, to an antigen presenting cell (APC). The targeting approach disclosed uses, for example, the major histocompatibility complex (MHC) class II binding motif from a superantigen coupled to a modulator of the Notch signalling pathway. Superantigens bind both MHC class II mols. and subsets of T cell receptors and thus effectively cross-link APCs to T cells and activate cells polyclonally. The mol. regions of these mols. that impart T cell receptor (TCR) and MHC class II binding have been defined structurally and have been shown to be distinct regions of the mol. By using the MHC class II binding domain with a modulator of the Notch signalling pathway we can focus the activity of the Notch signalling pathway modulator to the APCs at the site of delivery. Further, the domain lacks toxin activity because it cannot find the T cell receptor to activate T cells. According to one aspect of the present invention there is provided a conjugate comprising a first and a second sequence wherein the first sequence comprises a polypeptide which is capable of binding to an APC, or a polynucleotide encoding therefor, and the second sequence comprises a polypeptide comprising a modulator of a signalling pathway in a T cell or a polynucleotide encoding therefor.

L18 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN
2002:574952 Document No. 137:139357 Chimeric antibodies comprising **CD64**-binding human Fc and heterologous T cell epitopes for stimulating cytotoxic T cell response against pathogens and tumor. Durrant, Linda Gillian; Parsons, Tina; Robins, Adrian (Scancell Limited,

UK; Cancer Research Campaign Technology Limited). PCT Int. Appl. WO 2002058728 A2 20020801, 87 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB354 20020128. PRIORITY: GB 2001-2145 20010126.

AB The invention relates to the use of a polypeptide which comprises (i) a first portion comprising the part of human Fc which binds to **CD64**, and (ii) a second portion comprising one or more heterologous T cell epitopes for stimulating cytotoxic and helper T cell response. The polypeptide may be an antibody which may be used to stimulate an cytotoxic T cell response against pathogens and tumor cells in patients in need of such treatment.

L18 ANSWER 3 OF 24 MEDLINE on STN DUPLICATE 1

2002700232 Document Number: 22304954. PubMed ID: 12417885. High-level expression of immunoreactive recombinant cat allergen (Fel d 1): Targeting to antigen-presenting cells. Vailes Lisa D; Sun Amanda W; Ichikawa Kunio; Wu Zining; Sulahian Timothy H; Chapman Martin D; Guyre Paul M. (INDOOR Biotechnologies Inc, Charlottesville, VA 22903, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2002 Nov) 110 (5) 757-62. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Cat allergen Fel d 1 is a heterodimer encoded by 2 separate genes that has been difficult to produce as a fully immunoreactive molecule. OBJECTIVE: We sought to engineer recombinant (r) Fel d 1 with IgE and IgG antibody binding comparable with that of the natural allergen that could be targeted to antigen-presenting cells. METHODS: The rFel d 1 chains were coexpressed in baculovirus, either linked to the anti-**CD64** antibody H22 (rFel d 1 H22(+)) or alone (rFel d 1 H22 (-)). Binding of expressed allergens to mouse and human antibodies was compared with that of natural (n) Fel d 1 by means of enzyme immunoassay and antigen-binding and inhibition RIAs. Binding of rFel d 1 H22 (+) to the **CD64** receptor on leukocyte subpopulations and on the THP -1 cell line was analyzed by means of flow cytometry. RESULTS: The baculovirus-expressed allergens migrated with molecular weights of 49 kd (rFel d 1 H22(+)) and 22 kd (rFel d 1 H22 (-)). The rFel d 1 inhibited IgG antibody binding to nFel d 1 by greater than 95% and showed identical dose-dependent inhibition curves. There was an excellent quantitative correlation between IgE and IgG antibody binding to rFel d 1 and nFel d 1 in sera from patients with cat allergy (IgE: n = 258, r = > 0.72,P <.001). The rFel d 1 H22(+) bound to monocytes but not to lymphocytes or neutrophils, and binding of rFel d 1 H22(+) to THP-1 cells was inhibited by a soluble **CD64 fusion protein**.
CONCLUSIONS: Recombinant Fel d 1 chains have been successfully coexpressed as mature proteins with comparable immunoreactivities to nFel d 1. The rFel d 1 can be targeted to antigen-presenting cells through **CD64**. These constructs will facilitate structural studies of Fel d 1 and the development of improved allergy diagnostics and therapeutics.

L18 ANSWER 4 OF 24 MEDLINE on STN DUPLICATE 2

2001669005 Document Number: 21571693. PubMed ID: 11714794. Receptor modulation by Fc gamma RI-specific **fusion proteins** is dependent on receptor number and modified by IgG. Guyre C A; Keler T; Swink S L; Vitale L A; Graziano R F; Fanger M W. (Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756, USA.) JOURNAL OF IMMUNOLOGY, (2001 Dec 1) 167 (11) 6303-11. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The high-affinity IgG receptor, FcgammaRI (**CD64**), is constitutively expressed exclusively on professional APCs. Human FcgammaRI binds monomeric IgG with high affinity and is, therefore,

saturated in vivo. The binding of IgG to FcgammaRI causes receptor recycling, while Abs that cross-link FcgammaRI cause rapid down-modulation of surface FcgammaRI. Because studies performed in the absence of ligand may not be representative of FcgammaRI modulation in vivo, we investigated the ability of FcgammaRI-cross-linking Abs and non-cross-linking derivatives to modulate FcgammaRI in the presence and absence of ligand. In the absence of ligand mAb H22 and wH22xeGFP, an enhanced green fluorescent protein (eGFP)-labeled **fusion protein** of H22, cross-linked and rapidly down-modulated surface FcgammaRI on the human myeloid cell line, U937, and its high FcgammaRI-expressing subclone, 10.6. This effect was dependent on the concentration of **fusion protein** and the level of FcgammaRI expression and correlated with internalization of both wH22xeGFP and FcgammaRI, itself, as assessed by confocal microscopy. A single-chain Fv version, sFv22xeGFP, which does not cross-link FcgammaRI, was unable to modulate FcgammaRI in the absence of IgG. However, if ligand was present, treatment with either monovalent or cross-linking **fusion protein** led to intracellular receptor accumulation. These findings suggest at least two alternate mechanisms of internalization that are influenced by ligand and demonstrate the physiologic potential of FcgammaRI to transport a large antigenic load into APCs for processing. These studies may lead to the development of better FcgammaRI-targeted vaccines, as well as therapies to down-modulate FcR involved in autoimmune diseases.

L18 ANSWER 5 OF 24 MEDLINE on STN DUPLICATE 3
2001341635 Document Number: 21240417. PubMed ID: 11342441. Targeting of a B7-1 (CD80) immunoglobulin G **fusion protein** to acute myeloid leukemia blasts increases their costimulatory activity for autologous remission T cells. Notter M; Willinger T; Erben U; Thiel E. (Department of Hematology and Oncology, Freie Universitat Berlin, Universitatsklinikum Benjamin Franklin, Berlin, Germany.) BLOOD, (2001 May 15) 97 (10) 3138-45. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Transfection of tumor cells with the gene encoding the costimulatory molecule B7-1 (CD80), the ligand for CD28 and cytotoxic T lymphocye antigen-4 on T cells, has been shown to result in potent T-cell-mediated antitumor immunity. As an alternative approach, this study analyzed the costimulatory capacity of a human B7-1 immunoglobulin G (IgG) **fusion protein** targeted to the cell membrane of human acute myeloid leukemia (AML) blasts. Flow cytometric analysis revealed a low constitutive expression of B7-1 on human AML blasts (on average, 3.0 +/- 4.3%; n = 50). In contrast, the expression of B7-2 (CD86) was highly heterogeneous and higher in AML blasts of French-American-British classification types M4 and M5 (P <.0001). The B7-1 IgG **fusion protein** used in this study efficiently costimulated the proliferation of resting and preactivated T cells when immobilized on plastic. After preincubation with B7-1 IgG, specific binding of the **fusion protein** to the high-affinity Fcgammareceptor I (**CD64**) on leukemic cells was demonstrated and was found to increase the proliferation of both allogeneic and autologous T cells in costimulation experiments. Furthermore, targeting of B7-1 IgG to the tumor membrane resulted in increased proliferation of autologous remission T cells and had the potential to generate an enhanced redirected cytotoxic T-cell response against autologous AML blasts. In summary, the targeting of B7-1 IgG **fusion protein** described in this study represents a strategy alternative to gene therapy to restore the expression of the costimulatory molecule B7-1 on human AML blasts, thereby enhancing their immunogenicity for autologous T cells. This new approach may have implications for T-cell-mediated immunotherapy in AML.

L18 ANSWER 6 OF 24 MEDLINE on STN DUPLICATE 4
2001213273 Document Number: 21103279. PubMed ID: 11160307. Colocalization of Fc gamma RI-targeted antigen with class I MHC: implications for antigen processing. Guyre C A; Barreda M E; Swink S L; Fanger M W. (Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756, USA.) JOURNAL OF

IMMUNOLOGY, (2001 Feb 15) 166 (4) 2469-78. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The high-affinity receptor for IgG (**CD64** or FcgammaRI) is constitutively expressed exclusively on professional APCs (monocytes, macrophages, and dendritic cells). When Ag is targeted specifically to FcgammaRI, Ag presentation is markedly enhanced, although the mechanism of this enhancement is unknown. In an effort to elucidate the pathways involved in FcgammaRI targeting, we developed a model targeted Ag using enhanced green fluorescent protein (eGFP). This molecule, wH22xeGFP, consists of the entire humanized anti-FcgammaRI mAb H22 with eGFP genetically fused to the C-terminal end of each CH3 domain. wH22xeGFP binds within the ligand-binding region by its Fc end, as well as outside the ligand-binding region by its Fab ends, thereby cross-linking FcgammaRI. Confocal microscopy studies revealed that wH22xeGFP was rapidly internalized by the high-FcgammaRI-expressing cell line U937 10.6, but did not associate with intracellular proteins Rab4, Rab5a, or Lamp-1, suggesting that the targeted **fusion protein** was not localized in early endosomes, recycling vesicles, or lysosomes. Interestingly, wH22xeGFP was found colocalized with intracellular MHC class I, suggesting that FcgammaRI-targeted Ags may converge upon a class I processing pathway. These data are in agreement with studies in the mouse showing that FcgammaRI targeting can lead to Ag-specific activation of cytotoxic T cells. Data obtained from these studies should lead to a better understanding of how Ags targeted to FcgammaRI are processed and under what conditions they lead to presentation of antigenic peptides in MHC class I, as a foundation for the use of FcgammaRI-targeted Ags as vaccines.

L18 ANSWER 7 OF 24 MEDLINE on STN

2001477008 Document Number: 21411455. PubMed ID: 11520788. The Notch ligand, Delta-1, inhibits the differentiation of monocytes into macrophages but permits their differentiation into dendritic cells. Ohishi K; Varnum-Finney B; Serda R E; Anasetti C; Bernstein I D. (Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.) BLOOD, (2001 Sep 1) 98 (5) 1402-7. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Notch-mediated cellular interactions are known to regulate cell fate decisions in various developmental systems. A previous report indicated that monocytes express relatively high amounts of Notch-1 and Notch-2 and that the immobilized extracellular domain of the Notch ligand, Delta-1 (Delta(ext-myc)), induces apoptosis in peripheral blood monocytes cultured with macrophage colony-stimulating factor (M-CSF), but not granulocyte-macrophage CSF (GM-CSF). The present study determined the effect of Notch signaling on monocyte differentiation into macrophages and dendritic cells. Results showed that immobilized Delta(ext-myc) inhibited differentiation of monocytes into mature macrophages (CD1a+/-CD14+/-**CD64**+) with GM-CSF. However, Delta(ext-myc) permitted differentiation into immature dendritic cells (CD1a+CD14-**CD64**-) with GM-CSF and interleukin 4 (IL-4), and further differentiation into mature dendritic cells (CD1a+CD83+) with GM-CSF, IL-4, and tumor necrosis factor-alpha (TNF-alpha). Notch signaling affected the differentiation of CD1a-CD14+ macrophage/dendritic cell precursors derived in vitro from CD34+ cells. With GM-CSF and TNF-alpha, exposure to Delta(ext-myc) increased the proportion of precursors that differentiated into CD1a+CD14- dendritic cells (51% in the presence of Delta(ext-myc) versus 10% in control cultures), whereas a decreased proportion differentiated into CD1a-CD14+ macrophages (6% versus 65%). These data indicate a role for Notch signaling in regulating cell fate decisions by bipotent macrophage/dendritic precursors.

L18 ANSWER 8 OF 24 MEDLINE on STN DUPLICATE 5

2001074799 Document Number: 20569377. PubMed ID: 11119616. Functional and selective targeting of adenovirus to high-affinity Fcgamma receptor I-positive cells by using a bispecific hybrid adapter. Ebbinghaus C; Al-Jaibaji A; Operschall E; Schoffel A; Peter I; Greber U F; Hemmi S.

(Institute of Molecular Biology, University of Zurich, CH-8057 Zurich, Switzerland.) JOURNAL OF VIROLOGY, (2001 Jan) 75 (1) 480-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Adenovirus (Ad) efficiently delivers its DNA genome into a variety of cells and tissues, provided that these cells express appropriate receptors, including the coxsackie-adenovirus receptor (CAR), which binds to the terminal knob domain of the viral capsid protein fiber. To render CAR-negative cells susceptible to Ad infection, we have produced a bispecific hybrid adapter protein consisting of the amino-terminal extracellular domain of the human CAR protein (CARex) and the Fc region of the human immunoglobulin G1 protein, comprising the hinge and the CH2 and CH3 regions. CARex-Fc was purified from COS7 cell supernatants and mixed with Ad particles, thus blocking Ad infection of CAR-positive but Fc receptor-negative cells. The functionality of the CARex domain was further confirmed by successful immunization of mice with CARex-Fc followed by selection of a monoclonal anti-human CAR antibody (E1-1), which blocked Ad infection of CAR-positive cells. When mixed with Ad expressing eGFP, CARex-Fc mediated an up to 250-fold increase of transgene expression in CAR-negative human monocytic cell lines expressing the high-affinity Fcgamma receptor I (**CD64**) but not in cells expressing the low-affinity Fcgamma receptor II (CD32) or III (CD16). These results open new perspectives for Ad-mediated cancer cell vaccination, including the treatment of acute myeloid leukemia.

L18 ANSWER 9 OF 24 MEDLINE on STN DUPLICATE 6

2001196546 Document Number: 21126048. PubMed ID: 11223078. Exogenous antigen targeted to FcgammaRI on myeloid cells is presented in association with MHC class I. Wallace P K; Tsang K Y; Goldstein J; Correale P; Jarry T M; Schlom J; Guyre P M; Ernstoff M S; Fanger M W. (Department of Microbiology, HB7556, Dartmouth Medical School, 1 Medical Center Drive, Lebanon, NH 03756, USA.. pkw@dartmouth.edu) . JOURNAL OF IMMUNOLOGICAL METHODS, (2001 Feb 1) 248 (1-2) 183-94. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Vaccine therapy is attractive for prostate cancer patients because the tumor is slow growing (allowing time to augment host responses) and occurs in an older population less likely to tolerate more toxic treatments. We have constructed an expression vector based on a monoclonal antibody (mAb) that targets the high affinity receptor for IgG (FcgammaRI, **CD64**) which is exclusively expressed on myeloid cells including dendritic cells (DC). The heavy chain of mAb H22 CH2 and CH3 domains were removed and replaced with the gene for prostate specific antigen (PSA). Using that vector, we have constructed and purified FPH22.PSA, a **fusion protein** that targets PSA to FcgammaRI on antigen presenting cells (APC). This **fusion protein** has an apparent molecular mass of 80-83 kDa, binds to FcgammaRI with high affinity and expresses PSA. We demonstrate that FPH22.PSA targeted PSA was internalized and processed by the human myeloid THP-1 cell line resulting in presentation of MHC class I-associated PSA peptides and lysis of THP-1 by PSA-specific human CTL. Moreover, pretreatment of THP-1 cells with antibodies to block either FcgammaRI or MHC class I, blocked lysis indicating that targeting to FcgammaRI results in presentation of exogenous antigen on MHC class I molecules. These data demonstrate that FPH22.PSA was processed in such a manner by the myeloid cell line to allow for presentation of immunodominant peptides in MHC class I molecules and suggests that uptake of antigen via FcgammaRI results in cross-priming.

L18 ANSWER 10 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

2002:129947 Document No.: PREV200200129947. Membrane targeting of dimeric interleukin (IL)-2-immunoglobulin G1 induces cytotoxic T cell activity against autologous human acute myeloid leukemia (AML) blasts and prevents T cell apoptosis. Notter, Michael [Reprint author]; Erben, Ulrike [Reprint author]; Bittroff-Leben, Alexandra [Reprint author]; Thiel, Eckhard [Reprint author]. Hematology, Oncology and Transfusion Medicine, Benjamin Franklin Medical Center, Free University, Berlin, Germany. Blood,

(November 16, 2001) Vol. 98, No. 11 Part 1, pp. 122a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Tumor-induced T cell anergy is a limiting factor for the induction of an autologous transplant rejection-type antileukemic immune reaction in immunotherapy development. We tested the hypothesis that targeting IL-2 to the membrane of AML blasts and of immune effector cells induces a proliferative and cytotoxic T cell (CTL) response. Fusing the IL-2 gene to the hinge-, CH2-, and CH3-domain of human immunoglobulin (Ig) G1, and eukaryotic expression resulted in a dimeric IL-2-IgG1 molecule of 125 kDa. IL-2-IgG1 specifically bound to the high-affinity Fc-gamma receptor (R) I (**CD64**) of AML blasts. In the tumor membrane-bound state, IL-2-IgG1 increased proliferation of autologous remission T cells, and, in this respect, was superior to limiting concentrations of soluble IL-2. AML-M5 blasts coated with IL-2-IgG1, and CD8-IgG1 for control, were used as stimulator cells for autologous T cells. Despite the absence of soluble IL-2, IL-2-IgG1 presented on tumor cells on days 0 and 7 of culture, was sufficient to induce CTL activity on day 14 redirected against AML blasts by OKT3 present during the effector phase (21.5+-3.2% versus 7.8+-3.1% specific 51chromium-release at an effector:target ratio of 10:1). IL-2 R binding affinity of IL-2-IgG1 was 100-fold higher compared to IL-2. IL-2-dependent CTLL-16 cells were preincubated with saturating amounts of IL-2-IgG1 and monomeric IL-2. Following removal of excess cytokines by extensive washing, T cells coated with IL-2 did no longer incorporate 3H-thymidine after 24 hours, whereas IL-2-IgG1-pretreated CTLL-16 cells continued to proliferate (905+-72 versus 54.830+-4.652 cpm). Targeting IL-2-IgG1 but not IL-2 to CTLL 16 cells maintained their membrane asymmetry during this 24-hour culture period based on measuring phosphatidylserine exposure by annexin V binding using flow cytometry (13% versus 78% annexin V-positive cells). Furthermore, identical culture conditions followed by gel electrophoretic analysis revealed laddering of genomic DNA typical for apoptosis in the case of IL-2-pretreated CTLL-16 cells. In contrast, IL-2-IgG1 completely inhibited DNA fragmentation in this system indicating that its T cell-supporting activity relates to prevention of apoptosis. In conclusion, structural alterations of IL-2-IgG1 translated into novel biological effects creating potential to increase the immunogenicity of AML blasts for autologous T cells.

L18 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7
2002:418354 Document No.: PREV200200418354. A single chain Fv anti-**CD64**:ovalbumin **fusion protein** augments antigen presentation and results in higher IgG2a production. Sulahian, Timothy H. [Reprint author]; Sun, Amanda [Reprint author]; Symmes, Rebecca E. [Reprint author]; Goldstein, Joel; Wardwell, Kathleen [Reprint author]; Moser, Risha [Reprint author]; Guyre, Paul M. [Reprint author]. Department of Physiology, Dartmouth Medical School, Lebanon, NH, USA. Journal of Leukocyte Biology Supplement, (2001) No. 2001, pp. 75. print.
Meeting Info.: Joint Meeting of the Society for Leukocyte Biology and the International Cytokine Society: The Cytokine Odyssey 2001. Maui, HI, USA. November 08-11, 2001. Society for Leukocyte Biology; International Cytokine Society.
Language: English.

L18 ANSWER 12 OF 24 MEDLINE on STN
1999326334 Document Number: 99326334. PubMed ID: 10397749. The FcgammaRIa (**CD64**) ligand binding chain triggers major histocompatibility complex class II antigen presentation independently of its associated FcR gamma-chain. van Vugt M J; Kleijmeer M J; Keler T; Zeelenberg I; van Dijk M A; Leusen J H; Geuze H J; van de Winkel J G. (Departments of Immunology and Cell Biology, and Medarex Europe University Hospital Utrecht, Utrecht, The Netherlands.) BLOOD, (1999 Jul 15) 94 (2) 808-17. Journal code:

7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.
AB Within multi-subunit Ig receptors, the FcR gamma-chain immunoreceptor tyrosine-based activation motif (ITAM) plays a crucial role in enabling antigen presentation. This process involves antigen-capture and targeting to specific degradation and major histocompatibility complex (MHC) class II loading compartments. Antigenic epitopes are then presented by MHC class II molecules to specific T cells. The high-affinity receptor for IgG, hFcgammaRIa, is exclusively expressed on myeloid lineage cells and depends on the FcR gamma-chain for surface expression, efficient ligand binding, and most phagocytic effector functions. However, we show in this report, using the IIA1.6 cell model, that hFcgammaRIa can potentiate MHC class II antigen presentation, independently of a functional FcR gamma-chain ITAM. Immunoelectron microscopic analyses documented hFcgammaRIa alpha-chain/rabbit IgG-Ovalbumin complexes to be internalized and to migrate via sorting endosomes to MHC class II-containing late endosomes. Radical deletion of the hFcgammaRIa alpha-chain cytoplasmic tail did not affect internalization of rabbit IgG-Ovalbumin complexes. Importantly, however, this resulted in diversion of receptor-ligand complexes to the recycling pathway and decreased antigen presentation. These results show the hFcgammaRIa cytoplasmic tail to contain autonomous targeting information for intracellular trafficking of receptor-antigen complexes, although deficient in canonical tyrosine- or dileucine-targeting motifs. This is the first documentation of autonomous targeting by a member of the multichain FcR family that may critically impact the immunoregulatory role proposed for hFcgammaRIa (**CD64**).

L18 ANSWER 13 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:46827 Document No.: PREV200000046827. Dual signal targeting using a novel stem cell factor (SCF) 175-interleukin (IL)-2-myc His **fusion protein** and CD3 antibody against aberrant antigens of human acute myeloid leukemia (AML) blasts selectively stimulates T cells. Notter, Michael [Reprint author]; Erben, Ulrike [Reprint author]; Leben, Alexandra [Reprint author]; Bochert, Gudrun [Reprint author]; Thiel, Eckhard [Reprint author]. Hematology, Oncology and Transfusion Medicine, Universitaetsklinikum Benjamin Franklin, Berlin, Germany. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 79a. print.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L18 ANSWER 14 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:46828 Document No.: PREV200000046828. Targeting of a B7-1(CD80)IgG **fusion protein** to acute myeloid leukemia (AML) blasts increases their costimulatory activity for autologous remission T cells. Willinger, Tim [Reprint author]; Thiel, Eckhard [Reprint author]; Notter, Michael [Reprint author]. Abteilung fuer Haematologie und Onkologie, Universitaetsklinikum Benjamin Franklin, Berlin, Germany. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 79a. print.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

L18 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN
2000:908511 Document No. 135:44922 Targeting HIV-1 gp120 to the high affinity FC receptor (FC.gamma.RI, **CD64**) on myeloid antigen presenting cells: implications for enhancing vaccine responses. Howell, Alexandra L.; Thacker, Tara N.; Li, Fang; Fiering, Steve; Graziano, Robert F.; Goldstein, Joel; Fanger, Michael W. (V.A. Medical Center, VT, 05009, USA). Current Topics in Virology, 1, 61-70 (English) 1999. CODEN: CTVUAG. Publisher: Research Trends.
AB We prepd. a **fusion prot in** contg. a humanized monoclonal antibody (mAb), mAb H22, with specificity for the human high

affinity Fc receptor for IgG (Fc.gamma.RI, **CD64**), fused to HIV-1 gp120. This **fusion protein** construct was produced by joining the cDNA for the full length H22 heavy chain gene in frame to the cDNA for gp120. This construct, which also expressed a selectable marker, was stably transfected into a murine myeloma cell line that expressed the previously transfected H22 kappa light chain. The resulting **fusion protein**, (H22 .times. gp120), was secreted from the myeloma cell line and was purified by affinity chromatog. Flow cytometric anal. revealed that H22 .times. gp120 bound with high affinity via the Fab portion of H22 to **CD64** expressed on monocytes and macrophages from both humans and human **CD64**-expressing transgenic mice. Western blot anal. revealed that the 390 kDa **fusion protein** reacted with both anti-human IgG and anti-gp120 mAbs. Incubation of a monocyte cell line with this **fusion protein** at 37.degree.C resulted in internalization of the complex as detd. by flow cytometric anal. Immunization of human **CD64** transgenic mice with the purified H22 .times. gp120 **fusion protein** induced higher titers of anti-gp120 serum antibodies compared to immunization of non-transgenic littermates. Targeting gp120 to **CD64**-expressing antigen presenting cells (APC) in vivo may augment immune responses and enhance protective immunity.

L18 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1999:94795 Document No.: PREV199900094795. Targeting of A B7-1 (CD80) immunoglobulin G1 (IgG) **fusion protein** to acute myeloid leukemia (AML) blasts increases their costimulatory activity for autologous remission T cells. Willinger, T.; Thiel, E.; Notter, M.. Dep. Hematol. Oncol., Lab. Applied Cell. Mol. Immunol., Universitaetsklin. Benjamin Franklin, FU Berlin, Germany. Annals of Hematology, (1998) Vol. 77, No. SUPPL. 2, pp. S112. print.
Meeting Info.: Annual Congress of the German and Austrian Societies of Hematology and Oncology. Frankfurt, Germany. October 25-28, 1998. Austrian Society of Hematology and Oncology; German Society of Hematology and Oncology.
ISSN: 0939-5555. Language: English.

L18 ANSWER 17 OF 24 MEDLINE on STN DUPLICATE 8
97146059 Document Number: 97146059. PubMed ID: 8993006. Cytolytic and cytostatic properties of an anti-human Fc gammaRI (**CD64**) x epidermal growth factor bispecific **fusion protein**.
Goldstein J; Graziano R F; Sundarapandiyan K; Somasundaram C; Deo Y M. (Medarex, Inc., Annandale, NJ 08801, USA.) JOURNAL OF IMMUNOLOGY, (1997 Jan 15) 158 (2) 872-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB A bispecific **fusion protein** (H22-EGF) that binds simultaneously to the epidermal growth factor receptor (EGF-R) and to the high affinity receptor for the Fc portion of human IgG, Fc gammaRI (**CD64**), has been successfully constructed and expressed. For this construction, genomic DNA encoding the Fd fragment of humanized anti-Fc gammaRI mAb, H22, which binds Fc gammaRI at an epitope that is distinct from the Fc binding site, was fused to cDNA encoding human epidermal growth factor (EGF), a natural ligand for EGF-R. The resulting H22Fd-EGF-expressing vector was transfected into a myeloma cell line that was transfected previously with a vector containing DNA encoding the H22 kappa-light chain. SDS-PAGE analysis of purified H22-EGF demonstrated that the **fusion protein** was secreted predominantly as H22Fab'-EGF monomer (approximately 55 kDa), even though a free Cys residue exists in the hinge region of the H22 Fab' component. Using a novel bispecific flow cytometry-binding assay, we demonstrated that the purified bispecific **fusion protein**, H22-EGF, was able to bind simultaneously to soluble Fc gammaRI and EGF-R-expressing cells. H22-EGF inhibited the growth of EGF-R-overexpressing tumor cells and mediated dose-dependent cytotoxicity of these cells in the presence of Fc gammaRI-bearing cytotoxic effector cells. These results suggest that this

fusion protein may have therapeutic utility for EGF-R-overexpressing malignancies.

L18 ANSWER 18 OF 24 MEDLINE on STN DUPLICATE 9
1998098148 Document Number: 98098148. PubMed ID: 9435859. Increased potency of Fc-receptor-targeted antigens. Guyre P M; Graziano R F; Goldstein J; Wallace P K; Morganelli P M; Wardwell K; Howell A L. (Department of Physiology, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA.. paul.guyre@dartmouth.edu) . CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 146-8. Ref: 13. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A major challenge for using native and modified T cell epitopes to induce or suppress immunity relates to achieving efficient uptake and processing by antigen-presenting cells (APC) in vivo. IgG Fc receptors, which are expressed constitutively by professional APC including monocytes and dendritic cells, have long been known to mediate antigen uptake in a manner leading to efficient T cell activation. We have previously demonstrated enhanced presentation of antigenic and antagonistic peptides by targeting them to the type I Fc receptor for IgG (Fc gamma RI, **CD64**) on human monocytes. In the present report we review the literature suggesting that **CD64**-targeted antigens are likely to be effective in vivo, and present data demonstrating enhanced immunogenicity in **CD64** transgenic mice of a **fusion protein** that combines the specificities of HIV gp120 and the humanized anti-**CD64** monoclonal antibody H22. Overall, these studies suggest that targeting antigens to **CD64** represents an effective approach to enhancing the effectiveness of vaccines in vivo.

L18 ANSWER 19 OF 24 MEDLINE on STN DUPLICATE 10
1998098143 Document Number: 98098143. PubMed ID: 9435854. Targeting tumor cell destruction with **CD64**-directed bispecific **fusion proteins**. Graziano R F; Goldstein J; Sundarapandiyan K; Somasundaram C; Keler T; Deo Y M. (Medarex Inc., Annandale, NJ 08801, USA.. rgrazian@injersey.com) . CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 124-7. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L18 ANSWER 20 OF 24 MEDLINE on STN DUPLICATE 11
97060283 Document Number: 97060283. PubMed ID: 8903318. F(c)gammaRI-targeted **fusion proteins** result in efficient presentation by human monocytes of antigenic and antagonist T cell epitopes. Liu C; Goldstein J; Graziano R F; He J; O'Shea J K; Deo Y; Guyre P M. (Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1996 Nov 1) 98 (9) 2001-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB A major challenge for using native or modified T cell epitopes to induce or suppress immunity relates to poor localization of peptides to antigen presenting cells (APCs) in vivo. In this study, we demonstrate enhanced presentation of antigenic and antagonistic peptides by targeting them to the type I Fc receptor for IgG (F(c)gammaRI, **CD64**) on human monocytes. A Th epitope of tetanus toxoid, TT830, and the antagonistic peptide for TT830, TT833S, were genetically grafted into the constant region of the heavy chain of the humanized anti-**CD64** mAb 22 and expressed as monovalent **fusion proteins**, Fab22-TT830 and Fab22-TT833S. These **CD64**-targeted peptides were up to 1,000- and 100-fold more efficient than the parent peptides for T cell stimulation and antagonism, respectively, suggesting that such **fusion proteins** could effectively increase the delivery of peptides to APCs in vivo. Moreover, the F(c)gammaRI-targeted antagonistic peptide inhibited proliferation of TT830-specific T cells even when APCs were first pulsed with native peptide, a situation comparable with that which would be encountered in vivo when attempting to ameliorate an autoimmune response. These data suggest that targeted

presentation of antagonistic peptides could lead to promising Ag-specific therapies for T cell-mediated autoimmune diseases.

L18 ANSWER 21 OF 24 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1996:310083 Document No.: PREV199699032439. Presentation of agonist and antagonist T cell epitopes targeted to Fc-gamma-RI on human monocytes using anti-Fc-gamma-RI antibody-based **fusion proteins**.
Liu, C.; Graziano, R. F.; Goldstein, J.; He., J.; O'Shea, J.; Guyre, P. M.. Physiol. Dep., Dartmouth Med. Sch., Lebanon, NJ 03756, USA. FASEB Journal, (1996) Vol. 10, No. 6, pp. A1455.
Meeting Info.: Joint Meeting of the American Society for Biochemistry and Molecular Biology, the American Society for Investigative Pathology and the American Association of Immunologists. New Orleans, Louisiana, USA. June 2-6, 1996.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L18 ANSWER 22 OF 24 MEDLINE on STN
95138530 Document Number: 95138530. PubMed ID: 7836769. Interaction of human monocyte Fc gamma receptors with rat IgG2b. A new indicator for the Fc gamma RIIa (R-H131) polymorphism. Haagen I A; Geerars A J; Clark M R; van de Winkel J G. (Department of Immunology, University Hospital Utrecht, The Netherlands.) JOURNAL OF IMMUNOLOGY, (1995 Feb 15) 154 (4) 1852-60. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB Rat mAbs receive considerable interest for immunologic intervention in man. The rat IgG2b isotype has previously been found to be optimally active both in vivo and in vitro. We found that both a rat IgG2b CD3 mAb and a monovalent hybrid rat IgG2b-mouse IgG1 bispecific Ab triggered T cell activation in PBMC. Inhibition analyses with mAb blocking different human IgG Fc receptors (Fc gamma R) showed a dimorphic pattern. In donors expressing an Fc gamma RIIa-R/R131 allotype (previously defined on the basis of interaction with mouse (m) IgG1 as "high responder") anti-Fc gamma RI mAb 197 inhibited rat IgG2b induced T cell mitogenesis almost completely. In Fc gamma RIIa-H/H131 ("low responder" allotype) donors, however, both anti-Fc gamma RI mAb 197 and anti-Fc gamma RII mAb IV.3 were essential for optimal inhibition of mitogenesis. T cell proliferation experiments performed with the use of Fc gamma R-transfected fibroblasts as accessory cells showed the high affinity Fc gamma RIa (**CD64**) to interact with both rat IgG2b and rat IgG2b-mIgG1 hybrid CD3 mAb. The use of the two types of Fc gamma RIIa (CD32)-transfectants instead showed rat IgG2b CD3 mAb to interact solely with the IIa-H/H131 allotype. Interestingly, rat IgG2b-mIgG1 hybrid mAb did not interact effectively with this low affinity Fc gamma R. This suggests a requirement for only one rat IgG2b H chain for Fc gamma RIa-mediated binding, whereas two identical H chains seem to be necessary for proper interaction with Fc gamma RIIa. Ab-sensitized RBC-rosette experiments performed with the use of a rat IgG2b anti-NIP mAb confirmed the interaction pattern observed with rat CD3 mAb, supporting the phenomena to be isotype-, and not mAb-, dependent. These analyses point to a unique reactivity pattern for rat IgG2b Abs, interacting both with the high affinity Fc gamma RIa in all donors and Fc gamma RIIa of individuals expressing the IIa-H131 allotype.

L18 ANSWER 23 OF 24 MEDLINE on STN
96129466 Document Number: 96129466. PubMed ID: 8581368. A human Fc gamma RI/**CD64** transgenic model for in vivo analysis of (bispecific) antibody therapeutics. Heijnen I A; Van de Winkel J G. (Department of Immunology, University Hospital Utrecht, The Netherlands.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 351-6. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.
AB The human high-affinity IgG receptor, hFc gamma RI (**CD64**), is exclusively expressed on myeloid cells, where it serves an important role as a (cytotoxic) trigger molecule. To establish an in vivo model for analysis of the role of hFc gamma RI in immunity, we developed a novel transgenic mouse model. The human Fc gamma RIA gene, with endogenous regulatory sequences, was used to generate two lines of transgenic FVB/N

mice. Immunohistochemical and flow cytometric studies showed that hFc gamma RI expression was restricted to myeloid cells. Monocytes, macrophages, and polymorphonuclear neutrophils (PMN) expressed physiologic hFc gamma RI levels, whereas lymphocytes and mast cells lacked expression. Human Fc gamma RI expression was regulated in vivo by the cytokines IFN-gamma (exactly as in humans) and IL-10. The transgenic receptor proved functional and bound human tumor cells via anti-hFc gamma RI-based bispecific antibodies. hFc gamma RI could, furthermore, be efficiently targeted in vivo by **CD64** antibodies. These data demonstrate that the hFc gamma RI transgenic mouse model closely parallels the situation in humans. This mouse model seems useful for in vivo evaluation of the therapeutic potential of novel bispecific reagents in tumor and infection models.

L18 ANSWER 24 OF 24 MEDLINE on STN

92113247 Document Number: 92113247. PubMed ID: 1530954. Characterization of IgG FcR-mediated proliferation of human T cells induced by mouse and human anti-CD3 monoclonal antibodies. Identification of a functional polymorphism to human IgG2 anti-CD3. Parren P W; Warmerdam P A; Boeije L C; Capel P J; van de Winkel J G; Aarden L A. (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.) JOURNAL OF IMMUNOLOGY, (1992 Feb 1) 148 (3) 695-701. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB T cell activation induced by mouse anti-CD3 mAb has shown to be dependent on the Ig isotype of these antibodies. A study of isotype dependency of human antibodies, however, seems more relevant to human effector systems, especially in view of the availability of humanized antibodies for clinical applications. We constructed a panel of mouse and mouse/human chimeric anti-CD3 mAb, which differ only in their CH region and hence have identical binding sites and affinity. By using these antibodies, we now studied their ability to induce T cell proliferation in human PBMC and analyzed the classes of IgG FcR involved in these responses. The human (h)IgG1, hIgG3, and hIgG4, as well as mouse (m)IgG2a and mIgG3 anti-CD3 mAb induced an Fc gamma RI (**CD64**)-dependent T cell proliferation in all donors. Activation with hIgG2 and mIgG1 anti-CD3 mAb was observed to be mediated via the low affinity Fc gamma RII (CD32). It was found that leukocytes in a normal donor population display a functional polymorphism with respect to hIgG2 anti-CD3 responsiveness. This polymorphism was found to be inversely related to the previously defined Fc gamma RII-polymorphism to mIgG1 anti-CD3 mAb. Monocytes expressing the Fc gamma RII mIgG1 low responder (LR) allele support hIgG2 anti-CD3 induced T cell proliferation efficiently, whereas cells homozygous for the Fc gamma RII mIgG1 high responder (HR) allele do not. This observation could be confirmed in T cell activation studies using hFc gamma RIIa-transfected mouse fibroblasts, expressing either the mIgG1 anti-CD3 HR or LR Fc gamma RII-encoding cDNA.

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=> s CD40 fusion protein
L19          23 CD40 FUSION PROTEIN

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PROCESSING COMPLETED FOR L19
L20          11 DUP REMOVE L19 (12 DUPLICATES REMOVED)

=> d l20 1-11 cbib abs

L20  ANSWER 1 OF 11  CAPLUS  COPYRIGHT 2003 ACS on STN
2002:309880  Document No. 136:324055  Inhibiting B cell activation with
     soluble CD40 or fusion proteins thereof.  Aruffo, Alejandro A.; Ledbetter,
     Jeffrey A.; Stamenkovic, Ivan; Noelle, Randolph (Bristol-Myers Squibb
     Company, USA).  U.S. US 6376459 B1 20020423, 74 pp., Cont.-in-part of U.S.
     Ser. No. 835,799, abandoned.  (English).  CODEN: USXXAM.  APPLICATION: US
     1993-114944 19930831.  PRIORITY: US 1992-835799 19920214.
AB   The present invention relates to a counter-receptor, termed CD40CR, for
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the CD40 B-cell antigen, and to sol. ligands for this receptor, including fusion mols. comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a sol. CD40/Ig fusion protein or antibody specific for gp39 on T cells was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD protein receptor on helper T-cell membranes. The present invention provides for a substantially purified CD40CR receptor; for sol. ligands of CD40CR, including antibodies as well as fusion mols. comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation which may be esp. useful in the treatment of allergy or autoimmune disease, including graft-vs.-host disease and rheumatoid arthritis.

L20 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
2002:124388 Document No. 136:277753 Prolonged blockade of CD40-CD40 ligand interactions by gene transfer of CD40Ig results in long-term heart allograft survival and donor-specific hyporesponsiveness, but does not prevent chronic rejection. Guillot, Cecile; Guillonneau, Carole; Mathieu, Patrick; Gerdes, Christian A.; Menoret, Severine; Braudeau, Cecile; Tesson, Laurent; Renaudin, Karine; Castro, Maria G.; Lowenstein, Pedro R.; Anegon, Ignacio (Institut National de la Sante et de la Recherche Medicale, Institut de Transplantation et Recherche en Transplantation, Nantes, 44093, Fr.). Journal of Immunology, 168(4), 1600-1609 (English) 2002. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB Previous work on blockade of CD40-CD40 ligand interaction in mice and primates with anti-CD40 ligand mAbs has resulted in a moderate prolongation of allograft survival without the development of true allograft tolerance. In this study, the authors show in rats that adenovirus-mediated gene transfer of CD40Ig sequences into the graft resulted in prolonged (>200 days) expression of CD40Ig and in long-term (>300 days) survival. Recipients expressing CD40Ig displayed strongly (>90%) inhibited mixed leukocyte reactions and alloantibody prodn. at early (days 5 and 17) and late time points (>100 day) after transplantation, but showed limited inhibition of leukocyte infiltration and cytokine prodn. as evaluated by immunohistol. at early time points (day 5). Recipients of long-surviving hearts showed donor-specific hyporesponsiveness since acceptance of second cardiac allografts was donor specific. Nevertheless, long-term allografts (>100 days) displayed signs of chronic rejection vasculopathy. Occluded vessels showed leukocyte infiltration, mainly composed of CD4+ and CD8+ cells, macrophages, and mast cells. These recipients also showed anti-donor CTL activity. Recipients expressing CD40Ig did not show nonspecific immunosuppression, as they were able to mount anti-cognate immune responses that were partially inhibited at early time points and were normal thereafter. The authors conclude that gene transfer-mediated expression of CD40Ig resulted in a highly efficient inhibition of acute heart allograft rejection in rats. This treatment induced donor-specific inhibition of certain alloreactive mechanisms in the short-, but not the long-term, which resulted in long-term survival of allografts concomitant with the development of chronic rejection.

L20 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
2002:507152 Document No. 137:277621 Induction of donor-specific tolerance by adenovirus-mediated CD40Ig gene therapy in rat liver transplantation. Nomura, Masaru; Yamashita, Kenichiro; Murakami, Masaaki; Takehara, Megumi; Echizenya, Hayato; Sunahara, Masao; Kitagawa, Norihiko; Fujita, Miri; Furukawa, Hiroyuki; Uede, Toshimistu; Todo, Satoru (First Department of Surgery, Hokkaido University School of Medicine, Sapporo, Japan). Transplantation, 73(9), 1403-1410 (English) 2002. CODEN: TRPLAU. ISSN: 0041-1337. Publisher: Lippincott Williams & Wilkins.

AB Background. Blockade of CD40-CD40 ligand (CD154) costimulatory pathway with anti-CD154 antibody (Ab) prolongs allograft survival in exptl. organ transplantations; however, repeated agent administration is needed to provide an adequate immunosuppression. Seeking for simple and effective approach to interfere this signaling, we applied adenovirus-mediated gene

therapy by encoding CD40Ig gene (AdCD40Ig). Methods. Liver graft from ACI (RT1av1) rat was transplanted orthotopically into LEW (RT11) rat, and AdCD40Ig was given to animals via the penile vein immediately after grafting (n=6). Results. A single treatment with AdCD40Ig at 1 .times. 109 plaque forming units induced specific expression of CD40Ig gene on allograft liver, produced substantial amt. of the protein in the sera, and allowed indefinite graft survival. Whereas, LEW recipients given no treatment or control adenovirus vector (AdLacZ) promptly rejected ACI liver. In addn., AdCD40Ig-treated, long-term survivors accepted skin graft from the donor strain but not the third party graft. Histopathol. revealed that liver structure of the longterm surviving animals was completely preserved in normal with no infiltration of mononuclear cells. Conclusion. Blockade of CD40-CD154 pathway by CD40Ig gene therapy is a potent alloantigen-specific immunosuppressive strategy to induce permanent acceptance of liver allograft and would be a new therapeutic candidate in a clin. liver transplantation.

L20 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
2002:667788 Document No. 138:37662 Cloning and expression of the ovine CD40 molecule and the inhibition of the mixed lymphocyte reaction by the ovine CD40e-EGFP fusion protein. Zawitkowski, Madelyn; Russ, Graeme; Krishnan, Ravi (Basil Hetzel Institute, Transplantation Immunology Laboratory, The Queen Elizabeth Hospital, Woodville, 5011, Australia). Veterinary Immunology and Immunopathology, 89(1-2), 37-45 (English) 2002. CODEN: VIIMDS. ISSN: 0165-2427. Publisher: Elsevier Science B.V..
AB The CD40 mol. is a member of the tumor necrosis factor receptor (TNFR)-like supergene family and plays a major role as a co-stimulatory mol. in the activation of T cells in response to antigens presented by dendritic cells. In this study, reverse transcription-PCR cloning was used to derive the sequence encoding ovine CD40. The ovine CD40 sequence demonstrated a similarity of 97, 76 and 64% with the bovine, human and murine sequences, resp., at the nucleic acid level. The cysteine residues characteristic of the TNFR family and N-linked glycosylation sites are conserved. Furthermore, RNA anal. confirmed expression of CD40 mRNA in both ovine dendritic cells from lymphatic drainage and dermal fibroblasts in culture. In addn., cDNA encompassing the extracellular region of ovine CD40 (CD40e) was fused in-frame with the enhanced green fluorescent protein (EGFP) to generate a fusion protein upon the transfection of Chinese hamster ovary (CHO) cells. Immunopptn. with an anti-GFP monoclonal antibody of a 78 kDa a protein from conditioned medium of CHO transfectants confirmed that the CD40e-EGFP was secreted in the supernatant. All expts. were controlled with a pEGFP-N1 vector-blank construct. Moreover, the biol. activity of ovine CD40e-EGFP was demonstrated by its ability to inhibit a two-way mixed lymphocyte reaction. Thus these observations confirm that ovine CD40 blockade inhibits co-stimulation mediated by CD40-CD40L (CD154) interactions as has been reported in murine and human studies.

L20 ANSWER 5 OF 11 MEDLINE on STN DUPLICATE 1
2001209684 Document Number: 21195371. PubMed ID: 11298824. Rewiring of CD40 is necessary for delivery of rescue signals to B cells in germinal centres and subsequent entry into the memory pool. Siepmann K; Skok J; van Essen D; Harnett M; Gray D. (Department of Immunology, Imperial College School of Medicine, Hammersmith Hospital, London, UK.) IMMUNOLOGY, (2001 Mar) 102 (3) 263-72. Journal code: 0374672. ISSN: 0019-2805. Pub. country: England: United Kingdom. Language: English.
AB Memory B-cell development is impaired by in vivo blockade of the CD40-CD40 ligand (CD40L) interaction using human Fc immunoglobulin G1 (IgG1)-mouse **CD40 fusion protein** (CD40-Ig); however, germinal centre (GC) formation is not. We show here that the block in B-cell differentiation in these mice is at the stage of rescue from apoptosis and exit from the GC. Thus, GC from CD40-Ig-treated mice contain a three- to fourfold higher level of apoptotic cells than found in control mice injected with human IgG1 alone. This increase in apoptosis is not caused by a blockade of the CD40L-mediated rescue signal but is the

result of an intrinsic defect of GC B cells in CD40-Ig-treated mice to receive rescue signals via CD40. While anti-CD40 stimulation maintained the viability in culture of GC B cells from control mice, it did not rescue GC B cells from CD40-Ig-treated mice. This data is consistent with the notion that a 'rewiring' of the CD40 molecule is induced by CD40 ligation early in the response and is necessary to allow B-cell rescue from apoptosis when they subsequently enter the GC.

L20 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
2000:98349 Document No. 132:150607 Ex vivo treatment of allogeneic and xenogeneic T-cells with gp39 antagonists. Noelle, Randolph J.; Blazar, Bruce R.; Vallera, Daniel A.; Taylor, Patricia A. (Regents of the University of Minnesota, USA; Trustees of Dartmouth College). PCT Int. Appl. WO 2000006178 A1 20000210, 36 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US16686 19990729. PRIORITY: US 1998-124683 19980730.
AB Methods for inducing T-cell non-responsiveness to donor T-cells comprised in transplantation tissues, i.e. for inducing tolerance of donor T cell or for preventing graft-vs.-host disease, are provided. The methods involve ex vivo treatment of donor T-cells with gp39 antagonists, e.g. antibody or antibody fragment, sol. CD40, and sol. **CD40 fusion protein**.

L20 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
1999:719024 Document No. 131:350272 Human and mouse CD40 ligands and analogs and cDNAs encoding them and their preparation and use. Armitage, Richard J.; Fanslow, William C.; Spriggs, Melanie K.; Srinivasan, Subhashini; Gibson, Marylou G.; Morris, Arvia E.; McGrew, Jeffrey T. (Immunex Corporation, USA). U.S. US 5981724 A 19991109, 65 pp., Cont.-in-part of U.S. Ser. No. 249,189. (English). CODEN: USXXAM. APPLICATION: US 1995-477733 19950607. PRIORITY: US 1991-783707 19911025; US 1991-805723 19911205; US 1992-969703 19921023; US 1994-249189 19940524.
AB Membrane-bound and sol. forms of the cytokine CD40 ligand (CD40-L) of mouse and human are described and cDNAs encoding them are cloned and expressed. Expression constructs and host cells for manuf. of the protein are described. Deletion and substitution analogs of the ligand that retain specific binding to the extracellular binding region of a CD40 receptor are described. Antisense nucleic acids and antibodies to the protein that can be used to modulate its action are described. Cell lines with high levels of CD40-L were screened for using a **CD40 fusion protein** with Ig/Fc as an affinity label and the mouse thymoma line EL-4 identified as high in the ligand. An expression cDNA library in the mammalian expression vector pDC406 was screened using the fusion protein upon expression in CV1-EBNA cells. The mouse clone was used as a probe to obtain a clone from a human peripheral blood lymphocyte cDNA library in .lambda.gt10. CD40-L stimulates B cell proliferation, the synthesis of polyclonal Igs and the shedding of Fc.epsilon.RII (CD23 antigen) from B cells.

L20 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
1999:633252 Document No. 131:270955 Monoclonal antibodies to CD40 ligand, pharmaceutical composition comprising the same and hybridomas producing the same. Armitage, Richard J.; Fanslow, William C.; Spriggs, Melanie K. (Immunex Corporation, USA). U.S. US 5961974 A 19991005, 59 pp., Cont.-in-part of U.S. Ser. No. 969,703, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-249189 19940524. PRIORITY: US 1991-783707 19911025; US 1991-805723 19911205; US 1992-969703 19921023.
AB Disclosed is a polypeptide (CD40-L) and DNA sequences, vectors and transformed host cells useful in providing CD40-L polypeptides. More

particularly, this invention provides isolated human and murine CD40-L polypeptides that bind to the extracellular binding region of a CD40 receptor. The invention further provides CD40-L fragment for prodn. of monoclonal antibodies specific for CD40-L. Also, sol. CD40 protein and **CD40 fusion proteins** are prepd. for inducing B cell proliferation and antibody (e..g IgE) secretion, and for anti-allergic treatment. Also, fusion proteins comprising sol. human or murine CD40-L and Fc or trimeric CD40-L are constructed. In summary, CD40 agonists (i.e. membrane-bound CD40-L and oligomeric CD40-L) are provided for use as vaccine adjuvant and antibody prodn. stimulant, and CD40 antagonists (i.e. CD40 receptor, CD40/Fc, and sol. monomeric CD40-L) are provided for treating autoimmune diseases.

L20 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 2

94267169 Document Number: 94267169. PubMed ID: 7515910. Costimulation through CD28 enhances T cell-dependent B cell activation via CD40-CD40L interaction. Klaus S J; Pinchuk L M; Ochs H D; Law C L; Fanslow W C; Armitage R J; Clark E A. (Department of Microbiology, University of Washington, Seattle 98195.) JOURNAL OF IMMUNOLOGY, (1994 Jun 15) 152 (12) 5643-52. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Changes in T cell helper function were analyzed when anti-CD3-activated T cells were costimulated with mAbs to the CD28 receptor (anti-CD28). T cell-dependent B cell growth and differentiation were consistently augmented if anti-CD3 stimulated-T cells were simultaneously activated with anti-CD28. Although anti-CD28 enhanced IL-2 and IL-4 production, it did not increase B cell responses solely by augmenting production of soluble lymphokines. Anti-CD28 costimulation induced increases on T cells of CD40 ligand (CD40L), known to promote B cell proliferation and Ig secretion. Because anti-CD28 promoted T cell helper functions and expression of CD40L, we examined the dependence for CD40L during T cell-dependent B cell responses. Although soluble **CD40 fusion proteins** only partially inhibited T cell-dependent B cell activation, we found a strict requirement for CD40L expression at initiating B cell responses. Both CD40L expression and T cell help were blocked by cyclosporin A after TCR cross-linking, and, unlike T cell proliferation, both remained cyclosporin A sensitive during CD28 costimulation. In addition, anti-CD28 could not compensate for the T cell helper deficiency of hyper IgM syndrome patients who lack functional CD40L. Thus, anti-CD28-induced T cell help is delivered via a CD40L-dependent process. The fact that cross-linking CD40 on B cells promotes expression of the B7/BB-1 ligand for CD28 suggest T and B interactions may have a reciprocal amplification mechanism.

L20 ANSWER 10 OF 11 MEDLINE on STN DUPLICATE 3

94275366 Document Number: 94275366. PubMed ID: 7516404. Memory B cell development but not germinal center formation is impaired by in vivo blockade of CD40-CD40 ligand interaction. Gray D; Dullforce P; Jainandunsing S. (Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.) JOURNAL OF EXPERIMENTAL MEDICINE, (1994 Jul 1) 180 (1) 141-55. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB To study the role of the CD40-CD40 ligand interaction in the development of memory B cells and its level of action during primary antibody responses in vivo, mice were injected with a soluble **CD40 fusion protein** (sCD40-gamma 1), so as to block the interaction. The effects of the treatment on the primary antibody response were reminiscent of hyper-immunoglobulin M (IgM) syndrome (HIMG1): antigen-specific IgG responses were grossly inhibited whereas the IgM response was augmented severalfold. The latter observation suggests that there is a T-dependent, CD40 ligand-independent pathway of B cell activation that leads to IgM responses and that a significant component of the IgM in HIMG1 patients is derived from T-dependent responses. The secondary response was not readily blocked by sCD40-gamma 1 treatment, indicating a relative independence of CD40 ligation of antigen-experienced

B cells. The most striking finding from these studies is that the development of memory B cell populations (measured by adoptive transfer) is grossly impaired by administration of sCD40-gamma 1 during the early induction phase of the response. It is surprising that although the generation memory is diminished, there is no quantitative difference in the development of germinal centers. Whereas entry of B cells into the memory cell pathway is dependent on CD40 ligation, the clonal expansion of the potential memory precursors in germinal centers seems not to require a CD40 signal.

L20 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
1993:493523 Document No. 119:93523 Murine and human cytokine (CD40-L) which binds to CD40, and soluble CD40 and CD40 fusion molecules. Armitage, Richard J.; Fanslow, William C.; Spriggs, Melanie K. (Immunex Corp., USA). PCT Int. Appl. WO 9308207 A1 19930429, 79 pp. DESIGNATED STATES: W: AU, CA, FI, JP, KR, NO; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US8990 19921023. PRIORITY: US 1991-783707 19911025; US 1991-805723 19911205.

AB The title CD40-L mols. are disclosed, as are related DNA sequences, vectors, and transformed host cells. The murine and human CD40-L polypeptides bind to the extracellular binding region of a CD40 receptor. Also provided are a CD40/IgG1 Fc region fusion protein and a sol. CD40 protein (sCD40) comprising the extracellular portion of CD40; both the CD40/Fc and sCD40 can inhibit CD40-L or anti-CD40 monoclonal antibody-induced B-cell stimulation, interleukin-4-induced IgE stimulation, and interleukin-4-induced CD23 induction in B-cells. Construction is described of a CD40/Fc DNA for prodn. of a fusion protein for use in detecting cDNA clones encoding a CD40 ligand. Also described are selection of a cell line putatively expressing CD40-L, prepn. of a cDNA library for expression cloning of murine CD40-L, cross-species hybridization methodol. used to isolate a human CD40-L homolog, anti-allergy therapeutic effects of sCD40 and CD40/Fc fusion protein, etc. Interaction of CD40 with its ligand was evidently the principal mol. interaction responsible for T-cell contact-dependent induction of B-cell growth and differentiation to both antigen-specific antibody prodn. and polyclonal Ig secretion.

=> s CD154 fusion protein
L21 2 CD154 FUSION PROTEIN

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PROCESSING COMPLETED FOR L21
L22 1 DUP REMOVE L21 (1 DUPLICATE REMOVED)

=> d l22 cbib abs

L22 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
2001:39801 Document No.: PREV200100039801. CD40L (**CD154**) **fusion protein** with pulmonary surfactant protein D as a prototype for soluble multimeric TNF superfamily ligand molecules. Kornbluth, R. S. [Reprint author]; Kee, K. [Reprint author]; Truong, N. H. [Reprint author]. University of California San Diego and VA San Diego Healthcare System, La Jolla, CA, USA. FASEB Journal, (April 20, 2000) Vol. 14, No. 6, pp. A1162. print.
Meeting Info.: Joint Annual Meeting of the American Association of Immunologists and the Clinical Immunology Society. Seattle, Washington, USA. May 12-16, 2000.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

=> s CD56 fusion protein
L23 0 CD56 FUSION PROTEIN

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=> s CD56
L24       14263 CD56

=> s 24 and T cell epitope
   4 FILES SEARCHED...
L25         369 24 AND T CELL EPITOPE

=> s l25 and fuse?
L26           0 L25 AND FUSE?

=> s CD45
L27       22859 CD45

=> s l27 and CD45 fusion protein
L28           0 L27 AND CD45 FUSION PROTEIN

=> s cd54
L29        7885 CD54

=> s l29 and CD54 fusion protein
L30           0 L29 AND CD54 FUSION PROTEIN

=> s CD95 fusion
L31           1 CD95 FUSION

=> d l31 cbib abs

L31  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS on STN
2000:656273  Document No. 133:251002  Role of CD95 and caspase activity for
     endotoxin-associated hepatotoxicity and lethality.  Wanner, G. A.; Mica,
     L.; Hentze, H.; Kunstle, G.; Kolb, S.; Trentz, O.; Ertel, W. (Klinik fur
     Unfallchirurgie, Universitatsspital Zurich, Switz.).  Chirurgisches Forum
     fuer Experimentelle und Klinische Forschung 509-512 (German) 2000.  CODEN:
     CFEKA7.  ISSN: 0303-6227.  Publisher: Springer-Verlag.
AB   The aim of this study was to analyze the role of caspase activity and CD95
     for endotoxin-mediated hepatic microvascular injury and endotoxin-assocd.
     lethality.  C3H/HeN mice were i.v. administered LPS (E. coli; 10 mg/kg
     b.w.) in the presence or absence of the caspase inhibitor z-VAD-fmk or a
     neutralizing CD95 fusion protein (CD95-Fp).  Control
     animals received saline.  After 6 h animals (n = 6/group) underwent
     laparotomy under rompun/ketanest anesthesia and hepatic microcirculation
     was analyzed using intravital fluorescence microscopy, including quant.
     anal. of sinusoidal perfusion and leukocyte adherence in postsinusoidal
     venules.  Liver injury was assessed by measuring plasma AST and ALT
     levels.  Caspase-1-like and -3-like activities were measured using
     specific fluorometric assays.  Finally, a survival study was performed,
     comparing LPS-treated mice with mice that received z-VAD-fmk (n =
     9/group).  Hepatic microcirculation after LPS administration was
     characterized by severe sinusoidal perfusion failure and increased
     adherence of leukocytes to the venular wall at 6 h.  Repetitive
     administration of z-VAD-fmk inhibited sinusoidal perfusion failure and
     attenuated leukocyte accumulation in postsinusoidal venules.  LPS-induced
     increase of liver enzymes was decreased by z-VAD-fmk.  Neutralization of
     CD95 had no influence on any of these parameters.  Caspase-3-like
     activities were comparable in all groups.  In contrast, LPS induced an
     increase of caspase-1-like activity in liver tissue which was blocked by
     z-VAD-fmk but not by CD95-Fp.  All animals of the LPS group died within 24
     h while 7 out of 9 animals survived this time period after z-VAD-fmk
     treatment.  These data indicate that CD95-independent activation of
     caspases is a key event in LPS-assocd. hepatotoxicity.  Caspase inhibition
     may represent a new therapeutic concept to counteract endotoxin-mediated
     liver injury and lethality.

=> s B cell antigen
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L32 6510 B CELL ANTIGEN

=> s l32 and fusion
L33 197 L32 AND FUSION

=> s l33 and T helper epitope
L34 0 L33 AND T HELPER EPITOPE

=> dup remove l33
PROCESSING COMPLETED FOR L33
L35 116 DUP REMOVE L33 (81 DUPLICATES REMOVED)

=> s l35 and antibody response
L36 4 L35 AND ANTIBODY RESPONSE

=> dup remove l36
PROCESSING COMPLETED FOR L36
L37 4 DUP REMOVE L36 (0 DUPLICATES REMOVED)

=> d l37 1-4 cbib abs

L37 ANSWER 1 OF 4 MEDLINE on STN
2002352653 Document Number: 22090608. PubMed ID: 12096035. In vivo induction of tolerance by an Ig peptide is not affected by the deletion of FcR or a mutated IgG Fc fragment. el-Amine Moustapha; Hinshaw Jennifer A; Scott David W. (Department of Immunology, American Red Cross, J. Holland Laboratory, Rockville, MD 20855, USA.) INTERNATIONAL IMMUNOLOGY, (2002 Jul) 14 (7) 761-6. Journal code: 8916182. ISSN: 0953-8178. Pub. country: England: United Kingdom. Language: English.

AB To induce tolerance to a variety of epitopes, we have designed a gene therapy approach in which peptides or antigens are expressed in frame on a soluble IgG **fusion** protein scaffold and delivered via retroviral gene therapy in B cells in vivo. Initially, tolerance to the lambda repressor cI sequence p1-102 or its immunodominant epitopes (e.g. p12-26 or p73-88) was elicited in both T cells and B cells when lipopolysaccharide (LPS) blasts are transduced and injected into naive or even primed recipients. While a role of secreted Ig **fusion** protein in this process is not clear, we have previously demonstrated the importance of antigen presentation on MHC class II of **B cell antigen**-presenting cells (APC) for tolerance induction. To further examine the role of the Ig and especially of the Fc portion of the IgG in tolerogenesis, we transduced LPS blasts from FcR gamma II(-/-), Fc gamma RI(-/-), Fc gamma RIII(-/-), FcR(-/-) or naive mice with retroviral vectors expressing IgG1-102, Delta IgG1-102 (mutated construct on position 297 of the Fc portion) or IgG12-26. When these transduced LPS blasts from FcR knockout mice were injected into normal (or knockout) syngeneic recipient mice, they induced tolerance both to the immunodominant epitopes and the full-length protein in that the **antibody responses** to the immunodominant epitopes were reduced. In this paper, we show that this tolerance resides at both the T and B cell level. Moreover, mutation of residue 297, which affects IgG functions including FcR binding, did not alter the tolerogenicity of the construct. These results suggest that the Fc portion of the IgG molecules is not required for humoral nor for cellular tolerance induction using the IgG-antigen tolerogens.

L37 ANSWER 2 OF 4 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
2002:373544 The Genuine Article (R) Number: 547NR. Inducing specific reactivity against B cells in mice by immunizing with an Fc **fusion** protein containing self-Ig beta. Shen J J C; Huang J; Chang T W (Reprint) . Natl Tsing Hua Univ, Dept Life Sci, Hsinchu 300, Taiwan (Reprint). CANCER IMMUNOLOGY IMMUNOTHERAPY (MAY 2002) Vol. 51, No. 3, pp. 145-152. Publisher: SPRINGER-VERLAG. 175 FIFTH AVE, NEW YORK, NY 10010 USA. ISSN: 0340-7004. Pub. country: Taiwan. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A recombinant chimeric **fusion** protein, muIgbeta-hugamma4 .Fc, composed of the extracellular domain of mouse Igbeta (CD79b) and the CH2-CH3 domains of human IgGgamma4.Fc (hugamma4.Fc), linked via an immunologically inert flexible peptide, was prepared. The **fusion** protein was evaluated for its ability to induce specific auto-reactive immune response against Igbeta and to modulate B cell activity in Balb/c mice. Upon immunization with muIgbeta-Hugamma4.Fc, mice developed immunoglobulin (IgG) against selr-Igbeta, which could bind to the cells of a mouse B cell line expressing Igbeta on the cell surface. The proportion of B cells in mononuclear cells in the peripheral blood (PBMC) of treated mice decreased as compared to that of mice immunized with hugamma4.Fc without the Igbeta component. Furthermore, mice immunized against muIgbeta-hugamma4.Fc displayed a reduced **antibody response** against an irrelevant antigen. The implications of employing the present approach in developing a therapeutic strategy for regulating B cell activity has been discussed.

L37 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
1998:540542 Document No. 129:243940 Transient inhibition of CD28 and CD40 ligand interactions prolongs adenovirus-mediated transgene expression in the lung and facilitates expression after secondary vector administration. Wilson, Christopher B.; Embree, Lisa J.; Schowalter, David; Albert, Richard; Aruffo, Alejandro; Hollenbaugh, Diane; Linsley, Peter; Kay, Mark A. (Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, 98195, USA). Journal of Virology, 72(9), 7542-7550 (English) 1998. CODEN: JOVIAM. ISSN: 0022-538X. Publisher: American Society for Microbiology.

AB Recombinant adenovirus vectors have been used to transfer genes to the lungs in animal models, but the extent and duration of primary transgene expression and the ability to achieve expression after repeated vector administration have been limited by the development of antigen-specific immunity to the vector and, in some cases, to vector-transduced foreign proteins. To det. if focused modulation of the immune response could overcome some of these limitations, costimulatory interactions between T cells and **B cells/antigen**-presenting cells were transiently blocked around the time of vector administration. Systemic treatment at the time of primary-vector administration with a monoclonal antibody (MR1) against murine CD40 ligand, combined with recombinant murine CTLA4Ig and intratracheal coadministration of an adenovirus vector transducing the expression of murine CTLA4Ig, prolonged adenovirus-transduced .beta.-galactosidase expression in the airways for up to 28 days and resulted in persistent alveolar expression for > 90 days (the duration of the expt.). Consistent with these results, this treatment regimen reduced local inflammation and markedly reduced the T-cell and T-cell-dependent **antibody response** to the vector. A secondary adenovirus vector, administered >90 days after the last systemic dose of MR1 and muCTLA4Ig, resulted in alk. phosphatase expression at levels comparable to those seen with primary-vector administration. Expression of the secondary transgene persisted in the alveoli (but not in the airways) for up to 24 days (the longest period of observation) at levels similar to those obsd. on days 3 to 4. These results indicate that transient inhibition of costimulatory mol. interactions substantially enhanced gene transfer to the alveoli but was much less effective in the airways. This suggests that there are differences in the efficiency or nature of mechanisms limiting transgene expression in the airways and in the alveoli.

L37 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1986:123112 Document No.: PREV198681033528; BA81:33528. ANTIGENS APPARENTLY NONIMMUNOGENIC FOR XID-Y MICE CAUSE THE DEVELOPMENT OF SPECIFIC SPLENIC PLASMABLASTS AND OF **FUSION** PARTNERS FOR SP-2 CELLS GIVING HYBRIDOMAS THAT SECRETE ANTIBODIES. OHRINER W D [Reprint author]; CEBRA J J. DEP BIOL, UNIV PA, PHILADELPHIA, PA 19104, USA. European Journal of Immunology, (1985) Vol. 15, No. 9, pp. 906-913.
CODEN: EJIMAF. ISSN: 0014-2980. Language: ENGLISH.

AB Although xid/Y mice fail to make a detectable primary **antibody response** to a variety of antigens such as Type III pneumococcal capsular polysaccharide (SIII), 2,4,6-trinitrophenyl (TNP)-Ficoll, pneumococcal C carbohydrate, group A streptococcal vaccine and several kinds of related antigenic determinants such as phosphorylcholine (PC) and N-acetyl-glucosaminyl (GlcNAc), even when the latter are coupled to hemocyanin (Hy), they do show: (a) an antigen-dependent development of splenic B cells which can act as successful, productive **fusion** partners for SP2 cells giving hybridomas making monoclonal anti-SIII, anti-PC, anti-GlcNAc, anti-TNP, etc., and (b) an antigen-dependent appearance of antigen-binding plasmablasts in their spleens. The frequencies of specific B cells arising in xid/Y males with either of these properties are of the same order of magnitude as those found in immunocompetent xid/X female littermates. Further, both PC-Hy and GlcNAc-Hy prime xid/Y and xid/X mice for quantitatively and qualitatively similar secondary responses. All three of these expressions of a specific, primary response occur in xid/Y mice in the absence of any rise in circulating antibodies. The properties of successful, productive normal **fusion** partners leading to secretory hybridoma lines are unknown. Thus we cannot decide whether the antigen-binding plasmablasts that arise in xid/Y mice can also play the role or productive **fusion** partners. Neither do we know whether the development of specific IgM and IgG3 plasmablasts in xid/Y mice after antigen stimulation is an abnormality reflecting the xid mutation. It cannot be excluded that the development of productive **fusion** partners, of nonsecretory plasmablasts and of memory cells are all interrelated and reflect a process that also normally occurs in xid/X, X/X and X/Y mice following similar immunization regimens. It is tempting to speculate that such cells initially responding may lag in the development of normal secretory mechanisms and that the "transformed" **fusion** partner may complement this deficiency.

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=> s TNF receptor fusion
L38          157 TNF RECEPTOR FUSION

=> s l38 and T helper epitope
L39            0 L38 AND T HELPER EPITOPE

=> dup remove 138
PROCESSING COMPLETED FOR L38
L40           77 DUP REMOVE L38 (80 DUPLICATES REMOVED)

=> s l40 and autoimmune
L41            5 L40 AND AUTOIMMUNE

=> dup remove l41
PROCESSING COMPLETED FOR L41
L42            5 DUP REMOVE L41 (0 DUPLICATES REMOVED)

=> d l42 1-5 cbib abs

L42  ANSWER 1 OF 5  SCISEARCH  COPYRIGHT 2003 THOMSON ISI on STN
1999:913122   The Genuine Article (R) Number: 258GA. Present importance of
     direct immunologically based intervention strategies using anticytokines
     in rheumatoid arthritis.  Boehme M W J (Reprint); Gao I K. WILHELM
     FRESENIUS KLIN, KLIN INNERE MED & RHEUMATOL, AUKAMMALLEE 39, D-65191
     WIESBADEN, GERMANY (Reprint); UNIV HEIDELBERG, MED KLIN, ABT INNERE MED 4,
     D-69115 HEIDELBERG, GERMANY; KLIN RHEUMAKRANKE, D-55543 BAD KREUZNACH,
     GERMANY. ZEITSCHRIFT FUR RHEUMATOLOGIE (OCT 1999) Vol. 58, No. 5, pp.
     251-266. Publisher: DR DIETRICH STEINKOPFF VERLAG. PLATZ DER DEUTSCHEN
     EINHEIT 25, D-64293 DARMSTADT, GERMANY. ISSN: 0340-1855. Pub. country:
     GERMANY. Language: German.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB      Rheumatoid arthritis (RA) is a chronic inflammatory multisystemic
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autoimmune disease of unknown origin. RA is clinically characterized by recurrent inflammation of joints, synovialitis, progressive destruction of cartilage or bone tissue and multiorgan involvement. Today all established therapies of RA are still unable to stop or even cure the disease. In most cases these therapies can only reduce progression. Furthermore, these therapies have substantial side effects, which can contribute to the increased mortality of disease. Therefore more effective therapies with fewer side effects are needed. In this context direct immunological intervention strategies increasingly gained interest to inhibit proinflammatory cytokines.

In vivo and in vitro studies as well as experimental therapies documented the important role of the proinflammatory cytokines TNF alpha and IL-1 in RA. The therapy with TNF alpha-antibodies or receptor fusion proteins as well as IL-1 receptor antagonists proved to be clinically as well as immunologically highly effective as therapy of RA. The single dose treatment is associated with mild side effects only. In addition, trials using combined TNF alpha-antibody and methotrexate therapy gave promising results. However, potential severe side effects may occur after repeated therapy cycles or may be discovered after prolonged time of observation only (e.g., allergic re-actions, induction of autoantibodies or malignancies). Therefore, at present these therapy options can only be recommended for selected patients, who are included into controlled clinical trials. In addition, repeated courses of therapy seem to lead to reduced therapeutical efficacy (especially in TNF alpha-antibody therapy). Further controlled studies with cytokine antagonists should especially address these problems and focus in particular on potential inductions of autoantibodies or malignancies as well as on additional long-term side effects.

In contrast to direct inhibition of TNF alpha or IL-1 several further therapies indirectly influence these cytokines by interference with their synthesis or by alteration of the respective receptors. The importance of these therapeutical options has to be determined as well as the possibility of combination of established therapies with immunological intervention strategies.

L42 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
1998:351787 Document No. 129:40158 Suppression of TNF.alpha. and IL-12 in therapy. Feldmann, Marc; Malfait, Anne-Marie Aline Michel; Butler, Debra Maree; Brennan, Fionula Mary; Maini, Ravinder Nath (Kennedy Institute of Rheumatology, UK; Feldmann, Marc; Malfait, Anne-Marie Aline Michel; Butler, Debra Maree; Brennan, Fionula Mary; Maini, Ravinder Nath). PCT Int. Appl. WO 9822137 A1 19980528, 66 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-GB3151 19971117. PRIORITY: US 1996-749979 19961115.

AB Methods for treating and/or preventing a TNF.alpha.-mediated disease in an individual are disclosed. Also disclosed are compns. comprising a TNF antagonist and an IL-12 antagonist. The TNF.alpha. antagonist is an antibody or a TNF receptor/IgG fusion protein or thalidomide, and the IL-12 antagonist is an antibody or phosphodiesterase inhibitor, e.g. pentoxifylline or rolipram. TNF.alpha.-mediated diseases include rheumatoid arthritis, Crohn's disease, and acute and chronic immune diseases assocd. with transplantation.

L42 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1997:288272 Document No.: PREV199799587475. Inhibiting tumour necrosis factor (TNF) modulates chorioretinal infiltrating T cell phenotype in experimental **autoimmune** uveoretinitis (EAU). Dick, A. D. [Reprint author]; Robertson, M. [Reprint author]; Davidson, L. [Reprint author]; Isaacs, J.; Hale, G.; Waldmann, H.. Dep. Ophthalmol., Univ.

Aberdeen, Aberdeen, UK. Investigative Ophthalmology and Visual Science, (1997) Vol. 38, No. 4 PART 1-2, pp. S705.
Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology, Parts 1-2. Fort Lauderdale, Florida, USA. May 11-16, 1997.
CODEN: IOVSDA. ISSN: 0146-0404. Language: English.

L42 ANSWER 4 OF 5 MEDLINE on STN
95389752 Document Number: 95389752. PubMed ID: 7660686. [TNF inhibitors: a new therapeutic perspective in chronic inflammatory diseases in rheumatology?]. TNF-Inhibitoren: Eine neue therapeutische Perspektive bei chronisch-entzundlichen Erkrankungen in der Rheumatologie?. Fenner H. ZEITSCHRIFT FUR RHEUMATOLOGIE, (1995 May-Jun) 54 (3) 158-64. Ref: 14. Journal code: 0414162. ISSN: 0340-1855. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.
AB Interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF alpha) have been identified as important mediators of chronic immuno-inflammatory disease states such as rheumatoid arthritis. Effector cells are triggered by these cytokines to release molecules involved in synovitis and rheumatoid joint destruction, namely prostanoids, leukotrienes, adhesion molecules and metalloproteinases. Modifications of natural inhibitors of IL-1 and TNF alpha, which have been shown to maintain the homeostasis of the cytokine system, are now available by DNA technology. Monoclonal antibodies to TNF and the **TNF receptor fusion** proteins TNFR 55-IgG and TNFR 75-IgG are currently under clinical investigation in rheumatoid arthritis, inflammatory bowel disease and septic shock. Preliminary results from clinical trials in rheumatoid arthritis suggest that TNF inhibition represents a promising novel interventional strategy providing anti-inflammatory activity and inhibition of effector molecules of structural joint damage.

L42 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
1995:47249 Document No.: PREV199598061549. Inhibition of EAE by **TNF-receptor fusion** proteins. Baker, D. [Reprint author]; Butler, D.; O'Neill, J. K.; Turk, J. L. [Reprint author]; Feldmann, M.. Pathol. Dep., Royal Coll. Surgeons England, London, UK. Journal of Neuroimmunology, (1994) Vol. 54, No. 1-2, pp. 151.
Meeting Info.: IVth International Congress of Neuroimmunology. Amsterdam, Netherlands. October 23-27, 1994.
CODEN: JNRIDW. ISSN: 0165-5728. Language: English.

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=> s IL4R or interleukin 4 receptor fusion
L43          259 IL4R OR INTERLEUKIN 4 RECEPTOR FUSION

=> s l43 and T helper epitope
L44            0 L43 AND T HELPER EPITOPE

=> s l43 and Ig
L45           17 L43 AND IG

=> dup remove l45
PROCESSING COMPLETED FOR L45
L46            9 DUP REMOVE L45 (8 DUPLICATES REMOVED)

=> d l46 1-9 cbib abs

L46 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
2003:442698 Document No. 139:258978 The distinct gene expression profiles of
     chronic lymphocytic leukemia and multiple myeloma suggest different
     anti-apoptotic mechanisms but predict only some differences in phenotype.
     Zent, Clive S.; Zhan, Fenghuang; Schichman, Steven A.; Bumm, Klaus H. W.;
     Lin, Pei; Chen, James B.; Shaughnessy, John D. (Division of
     Hematology/Oncology, Central Arkansas Healthcare System and University of
     Arkansas for Medical Sciences, Little Rock, AR, 72205, USA). Leukemia
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Research, 27(9), 765-774 (English) 2003. CODEN: LEREDD. ISSN: 0145-2126. Publisher: Elsevier Science Ltd..

AB We compared gene expression in purified tumor cells from untreated patients with chronic lymphocytic (CLL) (n=24) and newly diagnosed multiple myeloma (MM) (n=29) using the Affymetrix HuGeneFL microarray with probes for approx. 6800 genes. Hierarchical clustering anal. showed that CLL and MM have distinct expression profiles (class prediction). Gene and protein expression (measured by flow cytometry) correlated well for CD19, CD20, CD23, and CD138 in CLL and MM, but not for **Ig** light chain, CD38 and CD79b in CLL, or CD45 and CD52 in MM. CLL and MM differentially expressed 18% of 130 apoptosis related genes, suggesting differences in mechanisms of cell survival.

L46 ANSWER 2 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

2001:785603 The Genuine Article (R) Number: 475QZ. Upon prolonged allergen exposure IL-4 and IL-4R alpha knockout mice produce specific IgE leading to anaphylaxis. Grunewald S M (Reprint); Teufel M; Erb K; Nelde A; Mohrs M; Brombacher F; Brocker E B; Sebald W; Duschl A. Univ Wurzburg, Dept Dermatol, Josef Schneider Str 2, D-97080 Wurzburg, Germany (Reprint); Univ Wurzburg, Klin & Poliklin Haut & Gechlechskrankheiten, Wurzburg, Germany; Univ Wurzburg, Zentrum Infekt Forsch, Wurzburg, Germany; Univ Wurzburg, Biozentrum, Wurzburg, Germany; Univ Calif San Francisco, Howard Hughes Med Inst, San Francisco, CA 94143 USA; Univ Calif San Francisco, Dept Med & Microbiol Immunol, San Francisco, CA 94143 USA; Univ Cape Town, Dept Immunol, Cape Town, South Africa. INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (AUG 2001) Vol. 125, No. 4, pp. 322-328. Publisher: KARGER. ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. ISSN: 1018-2438. Pub. country: Germany; USA; South Africa. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: IL-4 and IL-13 are key regulators in atopic disorders and both signal through the receptor chain **IL4R** alpha. IL-4 and IL-13 are also the only cytokines known to induce class switching to IgE. We sought to compare allergen-specific IgE responses and allergic reactivity of wild-type (wt) mice with IL-4(-/-) and IL-4R alpha (-/-) mice, which lack both IL-4 and IL-13 functions. Methods: BALB/c wt, IL-4(-/-) and IL-4R alpha (-/-) mice were immunized with ovalbumin intranasally or intraperitoneally and specific antibody titers were measured by ELISA. Bronchoalveolar lavage fluids and lung tissue were analyzed cytologically and histologically. Allergic reactivity was determined by active cutaneous anaphylaxis and anaphylactic shock. Results: wt mice immunized intranasally or intraperitoneally showed high titers of specific IgE 3 and 6 weeks after primary sensitization, resulting in cutaneous anaphylaxis and anaphylactic shock upon challenge. Intranasal sensitization resulted in airway eosinophilia and goblet cell metaplasia. In contrast, IL-4(-/-) and IL-4R alpha (-/-) mice showed no specific IgE after 3 weeks, but produced high titers after 6 weeks. At this time cutaneous anaphylaxis and anaphylactic shock could be induced as in wt mice, but lung pathology was absent. Conclusions: We conclude that upon long-term allergen exposure, alternative switch mechanisms independent of IL-4 and **IL4R** alpha may induce IgE but not asthma-like lung pathology. This may be relevant for the development of allergic disease, since long-term allergen exposure is a frequent condition during allergic sensitization. Copyright (C) 2001 S. Karger AG, Basel.

L46 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 1

2001495514 Document Number: 21429111. PubMed ID: 11543630. A second-generation association study of the 5q31 cytokine gene cluster and the interleukin-4 receptor in asthma. Kauppi P; Lindblad-Toh K; Sevon P; Toivonen H T; Rioux J D; Villapakkam A; Laitinen L A; Hudson T J; Kere J; Laitinen T. (Department of Medicine, Helsinki University Central Hospital, Helsinki, FI-00290, Finland.) GENOMICS, (2001 Sep) 77 (1-2) 35-42. Journal code: 8800135. ISSN: 0888-7543. Pub. country: United States. Language: English.

AB We have analyzed a dense set of single-nucleotide polymorphisms (SNPs) and

microsatellites spanning the T-helper cytokine gene cluster (interleukins 3, 4, 5, 9, and 13, interferon regulatory factor-1, colony-stimulating factor-2, and T-cell transcription factor-7) on 5q31 and the gene encoding the interleukin-4 receptor (**IL4R**) on 16p12 among Finnish families with asthma. As shown by haplotype pattern mining analysis, the number of disease-associated haplotype patterns differed from that expected for the 129Q allele polymorphism in IL13 for high serum total immunoglobulin (**Ig**) E levels, but not for asthma. The same SNP also yielded the best haplotype associations. For **IL4R**, asthma-associated haplotype patterns, most spanning the S411L polymorphism, showed suggestive association. However, these haplotypes consisted of the major alleles for the intracellular part of the receptor and were very common among both patients and controls. The minor alleles 503P and 576R have been reported to be associated with decreased serum IgE levels and changes in the biological activity of the protein, especially when inherited together. In the Finnish population, these two polymorphisms segregated in strong linkage disequilibrium. Our data support previous findings regarding L4R, indicating that 503P and 576R may act as minor protecting alleles for IgE-mediated disorders.

L46 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
2002:73106 Document No. 137:31163 Comparative analysis of gene transcription levels in two types of HIV-associated lymphomas. Nenasheva, V. V.; Maksimov, V. V.; Nikolaev, A. I.; Tarantul, V. Z. (Inst. Mol. Genetiki, RAN, Moscow, 123182, Russia). Molekulyarnaya Genetika, Mikrobiologiya i Virusologiya (4), 27-31 (Russian) 2001. CODEN: MGMVDU. ISSN: 0208-0613. Publisher: Izdatel'stvo Meditsina.
AB To characterize the mol. mechanisms of lymphoma formation in HIV-infected humans, a method of 2-stage subtractive cloning was used, which detects genes whose expression is increased in cells of one lymphoma in comparison with the other. Using this method, the authors detd. the spectrum of genes whose expression was increased in centroblastic non-Hodgkin's lymphoma in comparison with immunoblastic non-Hodgkin's lymphoma. Several gene groups were distinguished in this spectrum; their probable involvement in lymphomagenesis is discussed.

L46 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 2
2000080132 Document Number: 20080132. PubMed ID: 10614495. Functional implications for signaling via the **IL4R**/IL13R complex on bovine cells. Trigona W L; Brown W C; Estes D M. (University of Missouri, College of Veterinary Medicine, Department of Veterinary Pathobiology, Columbia 65211, USA.) VETERINARY IMMUNOLOGY AND IMMUNOPATHOLOGY, (1999 Dec 15) 72 (1-2) 73-9. Journal code: 8002006. ISSN: 0165-2427. Pub. country: Netherlands. Language: English.
AB IL-4 and IL-13 share a wide range of activities on monocytes, epithelial cells and B cells and thus play an important role in host defense. Many of these activities are not conserved among species as human, but not murine, B cells are thought to be responsive to IL-13. We previously demonstrated that human IL-13 is highly conserved at the nucleic acid level with a candidate bovine IL-13 cDNA homologue. Moreover, recombinant human IL-13 stimulates **Ig** secretion by appropriately activated bovine B cells. These studies have been extended to examining **Ig** class switching at both the protein and mRNA levels in addition to examining other markers of cellular activation. Our results suggest that IL-13 influences B cell differentiation by enhancing IgM, IgG1, and IgE production. IL-13 stimulation alone increases MHC class II expression and progression through cell cycle, although at lower levels in comparison to rboIL-4. The biology of the receptors for IL-4 and IL-13 is complex and raises several key questions with regard to IL-4-dependent and -independent mechanisms of host immunomodulation. Recent studies suggest that at least four chains are involved. These include the p140 IL-4 binding chain (IL-4Ralpha), the common gamma chain (gammac chain), IL-13 receptor alpha- chain (IL-13Ralpha-1) and the IL-13 receptor alpha-2 chain (IL-13Ralpha-2). We have recently cloned cDNAs for the bovine homologues of the IL-13Ralpha-1 and IL-4Ralpha chains and evaluated mRNA expression

for a variety of cell types following stimulation. The expression patterns and their implications for receptor chain utilization in signaling via these key TH2 signature cytokines will be discussed.

L46 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
1998:671741 Document No. 130:51150 Expression and ligand binding assays of soluble cytokine receptor-immunoglobulin fusion proteins. Brown, Steven J.; Becherer, Kathleen A.; Blumeyer, Kirsten; Kautzer, Curtis; Axelrod, Fumiko; Le, Huong; McConnell, Stephen J.; Whalley, Alice; Spinella, Dominic G. (Chugai Biopharmaceuticals, Inc., San Diego, CA, 92121, USA). Protein Expression and Purification, 14(1), 120-124 (English) 1998. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB We have developed a cloning vector for the expression of type I cytokine receptor, NO, extracellular domain (ECD)-mouse IgG1, Fc fusion proteins. The vector has a versatile polylinker that allows in-frame cloning of the receptor ECD with the mouse IgG1 sequence to encode a receptor ECD-IgG1 fusion construct. The receptor-IgG1 fusion proteins are transiently expressed in useful amts. following transfection of the expression vector into COS7 cells and G418 selection. The mouse IgG1 portion of the fusion protein provides a universal handle for purifn. on an affinity matrix and detection by anti-mouse IgG antibodies in ELISA or Western blot formats. The expressed receptor ECD-IgG1 fusion proteins bind their cognate ligands. In order to demonstrate that the fusion proteins have similar ligand binding affinities as the native receptors, the affinity consts. (Kd) for EPOR, TNFR, IL-4R, and IL-6R-IgG1 fusion proteins were measured by surface plasmon resonance and shown to be in good agreement with published values. The TNFR-IgG1 fusion protein was employed in a demonstration of a novel ELISA format for detecting cytokine receptor binding to cytokine. (c) 1998 Academic Press.

L46 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
1997:707929 Document No. 128:2777 Overexpression, purification, and use of a soluble human interleukin-4 receptor .alpha.-chain/**Ig**.gamma.1 fusion protein for ligand binding studies. Seipelt, Irene; Hoffmann, Silke H.; Schmidt, Jurgen; Engels, Joachim W.; Beckers, Thomas (Department of Cancer Research, ASTA Medica AG, Frankfurt / Main, Germany). Biochemical and Biophysical Research Communications, 239(2), 534-542 (English) 1997. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic.

AB The pleiotropic cytokine IL-4 transmits cellular signals mainly via the IL-4 receptor complex, with the .alpha.-chain as the high affinity binding subunit. Here the authors describe the overexpression of a sol. IL-4R .alpha.-chain (sIL-4R) as a fusion to **Ig** .gamma.1 heavy chain, consisting of the H-CH2-CH3 domains, in baby hamster kidney cells. The dimeric fusion protein named sIL-4R:E.gamma.1 was purified from culture supernatant by protein-A affinity chromatog., yielding up to 10 mg/L homogenous protein which was highly stable. The antibody-like features of the sIL-4R:E.gamma.1 fusion protein allowed immobilization on a biosensor matrix for surface plasmon resonance measurements by direct amine coupling as well as immobilization on microtiter plates coated with protein A for displacement binding. Kinetic parameters (kon and koff) for binding of IL-4 or the antagonistic mutant IL-4Y124D to the sIL-4R:E.gamma.1 fusion protein on the chip as detd. with the BIA-core instrument showed a high affinity binding with KD = 239 pM and KD = 148 pM, resp. The extremely high kon rate and the relatively slow koff rate for both ligands highlighted the limits of the BIAcore technol. The binding affinity as calcd. in displacement binding studies with biotinylated IL-4 was similar for IL-4 and IL-4Y124D (IC50=1.1 nM), thus offering a simple alternative for initial characterization of IL-4 mutants.

L46 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
1993:617398 Document No. 119:217398 Desensitization to specific allergens with interleukin-4 receptor-binding fusion protein. Waters, Cory Ann; Nichols, Jean C. (Seragen, Inc., USA). PCT Int. Appl. WO 9315766 A1 19930819, 42 pp. DESIGNATED STATES: W: AU, CA, FI, JP, KR, NO, NZ; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE.

(English). CODEN: PIXXD2. APPLICATION: WO 1993-US1034 19930204. PRIORITY: US 1992-832843 19920210.

AB A method is disclosed for desensitizing an animal to a particular antigen, wherein at or about a time of exposure of the animal to the allergen, a mol. is administered which specifically binds to interleukin-4 (IL-4) receptor expressed on a peripheral blood mononuclear cell (PBMC) of the animal, and is capable of decreasing the viability of the PBMC to which it binds. Thus, DAB389IL-4 (a fusion protein in which the receptor-binding domain of diphtheria toxin has been replaced by human IL-4) was prepd. with std. recombinant DNA methodol. DAB389IL-4 eliminated IgE secretion by B cells undergoing **Ig** class switching, but did not eliminate IgE secretion by B-cells (from an atopic patient) which had already undergone an **Ig** class switch.

L46 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN

1992:565256 Document No. 117:165256 Fusion proteins containing constant regions of immunoglobulins, their production and use. Lauffer, Leander; Oquendo, Patricia; Zettlmeissl, Gerd; Seed, Brian (Behringwerke A.-G., Germany; General Hospital Corp.). Eur. Pat. Appl. EP 464533 A1 19920108, 20 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE. (German). CODEN: EPXXDW. APPLICATION: EP 1991-110307 19910622. PRIORITY: DE 1990-4020607 19900628.

AB Sol. fusion proteins of human proteins that do not belong to the **Ig** family fused to **Ig** fragments, are described for use in diagnosis and therapy. The **Ig**-derived moiety makes these proteins are easy to purify by affinity chromatog. The presence of the **Ig** fragment does not appear to unfavorably affect the biol. activity of the human protein, so the fusion proteins could be used without removal of the **Ig** domain(s). Plasmids encoding fusion proteins between human IgG1 hinge, CH2, and CH3-contg. protein and human thromboplastin, IL-4 receptor, and erythropoietin were prepd., and the chimeric genes for the first two fusion proteins were expressed in COS cells. These proteins, purified with protein A-Sepharose, displayed the same biol. activity as the nonfused parent proteins.

=> s T helper epitope fusion protein
L47 1 T HELPER EPITOPE FUSION PROTEIN

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L47 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN

2001:489634 Document No. 135:88013 Mouse gene Her-2/neu (c-erbB2) polynucleotides and polypeptides, and uses thereof in pharmaceutical compositions and/or vaccines for treatment of breast cancer. Spies, A. Gregory (Corixa Corp., USA). PCT Int. Appl. WO 2001048205 A2 20010705, 52 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US35648 20001229. PRIORITY: US 1999-474382 19991229.

AB The invention provides the mouse Her-2/neu (c-erbB2) oncogene protein, a fusion protein comprising the Her-2/neu protein linked to a T helper epitope not present within the native mouse protein, and polynucleotides encoding said proteins. The invention also provides an expression vector contg. a polynucleotide encoding the mouse Her-2/neu protein, and a host cell transformed with said vector. The invention further provides a pharmaceutical compn. comprised of said polynucleotides, polypeptides, or antigen presenting cells (such as dendritic cells or macrophages) expressing the mouse Her-2/neu protein, and the use of said pharmaceutical compn. in inhibiting the development of cancer (such as breast cancer) in

a patient. Still further, the invention provides: (1) a vaccine comprised of said polynucleotides, polypeptides, or antigen-presenting cells expressing the Her-2/neu protein, and a non-specific immune response enhancer (adjuvant) that induces a predominantly type I response, and (2) for the use of said vaccine in inhibiting the development of cancer in a patient. Finally, the invention provides: (1) a method for removing tumor cells from a biol. sample which involves contacting sample with T cells that specifically react with the mouse Her-2/neu proteins, (2) a method for stimulating and/or expanding T cells specific for the Her-2/neu protein, which involves the use of said polynucleotides, polypeptides or antigen-presenting cells, and (3) use of isolated said T cell in treatment of cancer. The cDNA sequence, as well as the corresponding amino acid sequence of mouse Her-2/neu protein are disclosed.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	268.81	269.02

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-17.58	-17.58

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